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AUTOSOMAL PRODUCTS OF MEIOSIS ARISING FROM
RADIATION-INDUCED INTERCHANGE IN FEMALE

DROSOPHILA MELANOGASTER

by /

WILLIAM GLEN GIBSON

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Department of Zoology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date Aug 4/77

ABSTRACT

The present study was initiated with the view of achieving two goals:

- 1) to establish a suitable genetic assay system for measuring the frequency of spontaneous and induced structural and numerical aberrations of autosomes during meiosis in females and 2) to provide a better understanding of the mechanisms responsible for the production of the aberrant classes recovered.

By selective exclusion of all regular meiotic products this system enabled the recovery of large numbers of aberrant products. The multiplier system served as an internal dosimeter and provided an estimate of the population size from which the aberrancies arose which in turn provided a measure of the frequencies of each event. The four different classes of exceptional meiotic products were named according to the source or the structural nature of the chromosomes: reductional nondisjunction as "matroclinous"; equational nondisjunction as "equationals"; loss of chromosome 2 as "patroclinous"; and the attachment of homologous arms as "compounds".

The results suggest that two main factors affect the recovery of induced aberrations: of most importance is isosequentiality and of lesser importance is genetic background. The three classes of simultaneously recovered progeny (excluding equational nondisjunctions) arise from a common mechanism of induction; a mechanism which also accounts for free arm formation. The location of the breaks, the position of the chromatids and the method of reconstitution determine the type of aberration produced. The reconstitution of these breaks in aberrant ways are referred to as interchanges. Furthermore, it would appear that the reconstitutions are restricted in that euchromatic breaks attach to euchromatic breaks and heterochromatic to heterochromatic. Inter-

changes resulting from breaks on opposite sides of the centromeres of homologues result in the formation of non-sister compound chromosomes and from breaks on opposite sides of the centromeres of sister chromatids result in the formation of sister compound chromosomes. The interchange, if between heterologues, could lead to the nondisjunction of a pair of chromosomes and be recovered, as in the present study, as matroclinous progeny. The reciprocal product of the interchange between heterologues would produce an equal number of nullos eggs observed as patroclinous progeny, but if the dyad so formed is heteromorphic, i.e. chromatids of different length, it would result in the greater recovery of patroclinous progeny because of the preferential inclusion of the shorter chromatid. The evidence for interchange mediated aberrations is provided by the recovery of free arms of chromosome 2. Experimental support for these events is provided by the unequivocal identification of the centromeres involved, which, as in this study, is made possible through the use of metacentric autosomes.

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CHAPTER I

AN ASSAY SYSTEM TO RECOVER ABERRANT
CHROMOSOME-2 PRODUCTS OF MEIOSIS

INTRODUCTION

The present study was initiated with the view of achieving two goals: 1) to establish a suitable genetic assay system for measuring the frequency of spontaneous and induced structural and numerical aberrations of autosomes during meiosis in females, and 2) to provide a better understanding of the mechanisms responsible for the production of the aberrant classes recovered. The assay system developed in this study expanded beyond those previously used in that it provides for the recovery and, in most cases, the unambiguous identification of four different classes of aberrant chromosome-2 products of meiosis.

By selective exclusion of all regular meiotic products, this system facilitated the recovery of relatively large numbers of abnormal products. Furthermore, since the chromosome studied, chromosome 2, is a metacentric chromosome in which the left and right heterochromatic blocks have been characterized (Hilliker and Holm 1975; Hilliker 1976), I was able to obtain a more detailed analysis of events leading to the production of some of the aberrant chromosomal classes.

Parker (1968), Sobels (1971) and Abrahamson and Lewis (1971) have reviewed the various methods previously used to determine genetic damage in *Drosophila* and the advantages of using this organism. As indicated by Parker (1968), studies may fail to detect induced genetic damage not because it did not occur, but because an appropriate selective system, which avoided dominant lethality, was not available. This problem was more readily resolved with the sex chromosomes than with the autosomes and for this reason most of the assay systems thus far developed have focused on the X

and/or Y chromosome (Valencia 1970).

Early studies with the X chromosome demonstrated its potential use in assay systems for detecting various types of irregular meiotic events. Bridges (1916) first demonstrated abnormal X chromosome segregation in females. These flies produced female progeny that inherited both X chromosomes from their mother, and male progeny that received the X chromosome from their father. These exceptional progeny were called matroclinous and patroclinous respectively. Subsequent genetical and cytological analysis revealed that the mothers of these irregular progeny carried a free Y chromosome. Bridges (1916) believed that the Y chromosome caused the X's to nondisjoin. Thus, some eggs received both X chromosomes (disomic X) while others received none (nullo X). The former would give rise to matroclinous progeny when fertilized by a Y-bearing sperm, and the latter would give rise to patroclinous progeny when fertilized by an X-bearing sperm. Muller and Altenburg (1919), also using sex chromosomes bearing specific dominant genetic markers, were able to detect spontaneous recessive point mutations in females by the inviability of the hemizygous males that inherited the recessive lethals. Mavor (1924) and Muller (1927) subsequently showed that X-rays increased both the rate of nondisjunction and the frequency of recessive lethals. Although Mavor (1924) observed the increased rate of nondisjunction with X-rays, the results were not as he anticipated. Because formation of a nullo-X gamete was the reciprocal event to the formation of a disomic-X gamete, matroclinous and patroclinous progeny were expected in equal numbers. However, in his results the frequency of exceptional males was always greater than that of exceptional females. He concluded from this that patroclinous progeny were the sum of two independent events: 1) nondisjunction and 2) chromosome loss. Thus, the two phenomena have been studied concomitantly. Since the matro-

clinous female progeny represented nondisjunctional events, the numerical difference between the patroclinous and matroclinous progeny was defined as an index of chromosome loss.

The study of aneuploids, including nondisjunction and chromosome loss, has gained increased interest because surveys show 30 - 40 percent of all cases of spontaneous abortion in humans arise from nondisjunction and 0.4 percent of live-born children exhibit aneuploidy (Jacobs et al. 1959; Jacobs 1971).

At the same time studies were being conducted on nondisjunction, Morgan (1922) obtained results in sex linkage studies that could only be explained by the presence of two X chromosomes attached to a common centromere. Cytological examination confirmed her genetic prediction. Shortly thereafter, Anderson (1925) generated an attached-X chromosome which carried arms heterozygous for a series of genetic markers. Thus, for the first time, half-tetrad analysis was available in *Drosophila*. In the years that followed, various arrangement of attached X's were recovered. Novitski (1954) described these various attachments and gave them the general name of compound-X chromosomes.

Although much attention has been given to reductional nondisjunction, little effort has been directed at equational, or second division, nondisjunction. The reasons for this are inherent in the nature of the X chromosome. The X chromosome being acrocentric does not have euchromatin on both sides of the centromere. This makes genetic analysis of events in the heterochromatic regions ambiguous. Mavor (1924) and Anderson (1931) observed putative equational nondisjunctions of the X chromosome based on the recovery of progeny homozygous for a proximal marker from heterozygous female parents. They proposed that at meiosis II the two sister chromatids nondisjoined pro-

ducing a disomic gamete. The same results could be explained by a crossover between the proximal marker and the centromere, followed by a reductional nondisjunction. The lack of a method for detecting a crossover in the proximal region of an acrocentric chromosome makes it impossible to differentiate between a crossover followed by a reductional nondisjunction and an equational nondisjunction.

Similarly, the analysis of the centromere involved in a half translocation, involving the X chromosome, is uncertain because of the inability to genetically define heterochromatic events in an acrocentric chromosome. This is of particular concern since most radiation induced events occur in the heterochromatic region (Abrahamson, Herskowitz and Muller 1954; Parker and McCrone 1958).

The majority of studies in female *Drosophila* concerned with numerical and structural chromosomal aberrations, such as nondisjunction, both reductional and equational, chromosome loss and translocations, have involved the X chromosome. In most cases it has been difficult, if not impossible, to study these events in the major autosomes (either the second or the third chromosomes). Events similar to those which occur in the X chromosome were thought to take place in the autosomes, but until recently they had been undetectable owing to the lack of a rescue system which compensated for the aneuploidy produced by the event. Although nondisjunction of the second or third chromosome might occur in females, the resulting zygote would be lethal without sperm that were genetically complementary.

With the advent of compound autosomes, attempts have been made to study autosomal aberrancies. The first compound autosomes were constructed in the laboratory of E.B. Lewis (Rasmussen 1960; for a full description of the pro-

cedure followed in generating the original compound autosomes, see Holm 1976). Compound autosomes are formed by the attachment of two left, or two right arms of the second (or third) chromosome to a common centromere. Thus in a compound-2 stock, the pair of standard homologous second chromosomes would be replaced by a pair of heterologues, namely a compound-2L and a compound-2R. The meiotic properties of compound chromosomes, which differ in males and females, have provided us with an important genetic tool for studying exceptional meiotic behaviour of autosomes in *Drosophila*.

Consistent with the theory of distributive pairing (Grell 1962, 1964) in the absence of heterologous rearrangements, the compound-left usually segregates from the compound-right autosome during meiosis in females (Holm, Deland and Chovnick 1967; Grell 1970; Holm and Chovnick 1975; Holm 1976). By contrast, in males the complementary pair of compound autosomes assort independently, thereby generating, in almost equal proportions, four classes of sperm (Scriba 1967, 1969; Holm, Deland and Chovnick 1967; Clark and Sobels 1973; Lutolf 1972; Holm and Chovnick 1975; Holm 1976). With specific reference to compound seconds the following four meiotic products would be obtained: sperm carrying C(2L), sperm carrying C(2R), disomic-2 sperm and nullosomic-2 sperm (Figure 2). The latter two classes of sperm are those which serve to rescue meiotic products from females arising from chromosome-2 nondisjunction and loss. In addition, the first two classes of sperm provide the means of recovering newly generated C(2L) and C(2R) chromosomes.

Bateman (1968) was the first to report a study on autosomal nondisjunction in which the above approach was adopted. He mated C(2L);C(2R) males to irradiated females with standard chromosomes. The randomness of segregation of compound autosomes in males allowed both the recovery of nullosomic

(chromosome loss) and the recovery of disomic eggs (nondisjunction). He also recovered viable progeny through the formation of new C(2L) or C(2R) chromosomes. With one exception, these progeny derived one paternal compound and one newly generated compound from the female parent. There is, however, one major disadvantage to this system of recovery; it is not possible to obtain a direct measure of the frequencies of exceptional events as all regular eggs are inviable when fertilized by sperm from compound-2 males. Consequently, previous studies have provided only relative comparisons of the recovery of exceptional classes.

This first chapter contains the details of the multiplier system, a description of those experimental results that serve to support the adoption of such a system and the estimated frequencies of the four classes of aberrant products recovered from every experiment in which gamma radiation was used as the mutagenic agent and in which the exceptional events occurred spontaneously. The multiplier system served not only to estimate the size of the population from which the exceptional autosomal products were recovered but also as an internal dosimeter to provide an independent measure of the mutagenic effectiveness of the inducing agent. The internal dosimeter was obtained by carrying out an X-linked recessive lethal test on a proportion of the parental females in the multiplier system. While one might justifiably argue that such a precaution is not required in studies on radiation mutagenesis, its inclusion was viewed as absolutely essential to those studies in which the mutagenic properties of chemical agents are to be established. Therefore, all possible measurements were made on the effects of radiation to provide a wide scope of comparisons for studies on the effects of chemical mutagens.

MATERIALS AND METHODS

Description Of Chromosomes: The study of radiation induced chromosomal aberrations was made on chromosome 2 of *Drosophila*. Two chromosomes of standard sequence were used: one carried the genetic marker, 'ap^{wow}' (apterous-wow) and the other carried the markers, lt pk cn (light, prickle and cinnibar). The above two chromosomes were examined in combination with homologues of two structurally different types: a structurally homozygous unmarked standard-second chromosome (designated +) and two homologues that were structurally heterozygous owing to multiple inversions, In(2LR)S^Ml,Cy (Cy, curly) and In(2L + 2R)Cy. Both of these inverted chromosomes served as almost complete balancers for the second chromosome.

The males used in all experiments were of two types: 1) those used in the multiplier system, which also served as the internal dosimeter, had standard seconds and the inverted X-chromosome In(1)sc^{S¹L⁸R⁺}sc^{S¹L⁸a^B} (sc^{S¹}, scute of Sinitakaya; sc⁸, scute-8; w^a, white apricot; B, bar) which will be abbreviated Basc throughout the thesis, 2) those used to recover eggs that were aberrant for meiotic products of chromosome 2 carried compound-2 chromosomes taken from two different strains: the C(2L)VH2,lt;C(2R)P,px (lt, light; px, plexus) strain or the C(2L)P,b;C(2R)P,px (b, black; px, plexus) strain.

For a complete description of all genetic markers and rearranged standard chromosomes consult Lindsley and Grell (1968). The terminology used in describing compound autosomes is discussed by Holm and Chovnick (1975) and Holm (1976).

Radiation Treatment: Virgin females were collected over a 24 - 48 hour

period. During the collection period, the virgin females were stored at 17°C on food without a live yeast supplement. Experimental females were etherized, placed in shell vials and treated with 2000 rads of gamma radiation from a ^{60}Co source in the Department of Chemistry at the University of British Columbia. The dose rates of gamma radiation from the ^{60}Co source were periodically monitored by the Chemistry Department and were within an error of approximately $\pm 3\%$. Control females were handled in the same manner excluding treatment.

Mating Procedure For Experimental And Dosimetry Test: In each experiment approximately 1,100 females were treated. For studies on induced chromosomal aberrations one thousand of the treated females were mated singly in shell vials to two males bearing compound-2 chromosomes. Females with the ap^{wow} chromosome were mated to C(2L)VH2,lt;C(2R)P,px males while those females carrying the lt pk cn chromosome were mated to males carrying C(2L)P,b;C(2R)P,px. The two different strains of compound-2 males were selected to facilitate the identification of newly generated compound autosomes. The parents were left in the vials for four days before being cleared. Progeny were scored daily for eleven days following the first eclosions.

The remaining 100 females from each treatment were placed singly in shell vials each of which contained two Basc males. All vials were cleared after four days. The total number of progeny recovered from each mating was counted, and from each vial ten virgin females heterozygous for the genetic marker B, were again single pair mated to two Basc males. This resulted in 1,000 chromosomes being tested in each experiment. The criterion for classification of a recessive lethal was the absence of wild type males in the F2 generation. Any series of ten vials which had 30% or more without wild type

males was classified as a premeiotic event and thus not included in the calculation of the recessive lethal frequency.

An outline of the mating procedure both for the experimental crosses and for the dosimetry tests is presented in Figure 1.

Recovery Of Chromosomal Aberrations: The C(2L);C(2R) males gave rise to four classes of sperm and each class was assumed to be produced in equal frequency: nullo-2, disomic-2, C(2L) bearing and C(2R) bearing sperm (Figure 2). Since these sperm were not complementary to any normal egg with regards to the second chromosome, all normal oocytes were lethal. The only recoverable oocytes were those containing certain aberrant meiotic products of chromosome 2. These included: reductional or equational nondisjunction (disomic-2 eggs) which were recovered by a nullo-2 sperm; nullo-2 eggs, recovered by disomic-2 sperm; and new compound-2 chromosomes, either left or right, which were rescued by sperm carrying a complementary compound-2 chromosome. In this study the four different classes of exceptional meiotic products rescued in the progeny were named according to the source or structural nature of the chromosomes in the following manner: reductional nondisjunction (disomic-2 eggs) as "matroclinous", equational nondisjunction (disomic-2 eggs homozygous for sister-chromatid markers) as "equationals"; loss of chromosome-2 (nullo-2 eggs) as "patroclinous"; and newly generated compounds either as C(2L) or as C(2R). This method of nomenclature avoided any inference as to the mechanism responsible for the production of irregular meiotic events.

Multiplier System And Internal Dosimeter: As only exceptional meiotic products were recovered, it was necessary to have an estimate of the size of the zygotic population from which the exceptions were obtained in order to

FIGURE 1

The experimental mating procedure that was followed to yield exceptional progeny and to provide an estimate, via the multiplier system, of the population size from which the exceptions arose. As an extension of the multiplier system an X-linked recessive lethal test was conducted as an internal dosimeter for the effectiveness of the mutagens. *Base* males carry the balancer *X* chromosome $In(1)sc^{SL}sc^{8R}+S,sc^{SL}sc^{8}w^aB$.

EXPERIMENTAL MATING PROCEDURE

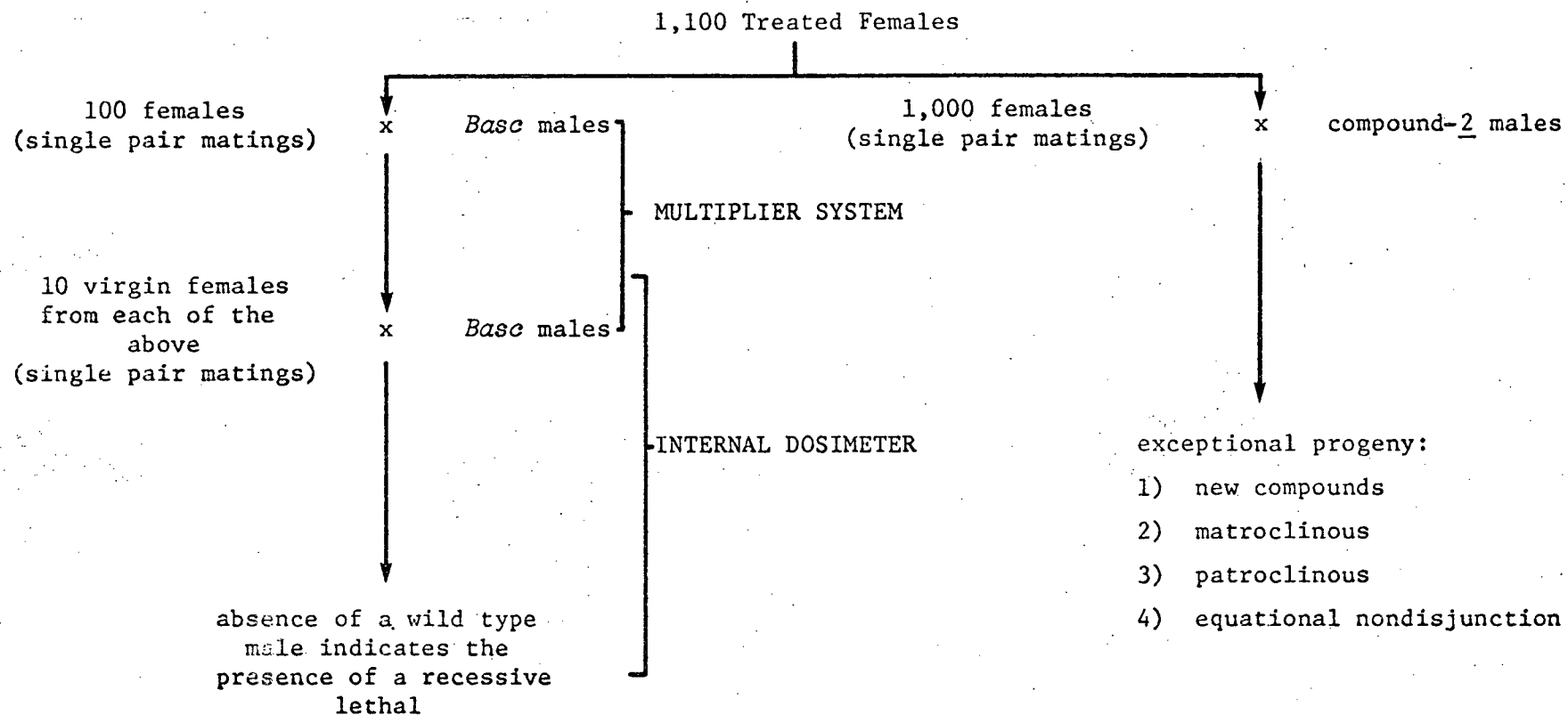



FIGURE 2

Males carrying compound-2 chromosomes produce four classes of sperm in approximately equal frequencies. The only recoverable female gametes are either those resulting from irregular behaviour of the chromosomes during meiosis or those containing a newly generated compound-2 autosome, thereby producing the four classes of progeny.

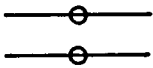
Gametes from Compound-2 Males

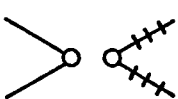
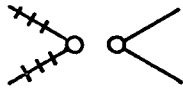
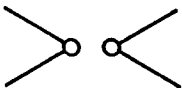
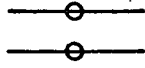
Recoverable Aberrant Female Gametes


New C(2R)


New C(2L)

Nullo - 2


Disomic-2

	C(2L)	C(2R)	C(2L);C(2R)	Nullo - 2
 new C(2R)				
 new C(2L)				
			 patro-clinous	
				 matro-clinous

determine the frequencies of a particular event. This was achieved by counting all progeny recovered from those matings in which (approximately 100) females were crossed to Basc males for the X-linked recessive lethal (dosimetry) test. The mean value of progeny from these vials gave an estimate of the average number of progeny expected from each experimental vial.

To test the assumption that compound autosomes assort independently during meiosis, C(2L)P,b;C(2R)P,px females were single pair mated to C(2L)VH2,lt;C(2R)P,px males in shell vials containing charcoal colored medium. The parents were transferred through five 24 hour broods. Each vial was examined for the presence of hatched and unhatched eggs. To avoid including any unfertilized eggs, the count was initiated only when the previous vial had also contained hatched eggs.

The use of a multiplier system implied that the number of eggs laid by the females were independent of the male used. Therefore, the number of eggs laid by In(2L + 2R)Cy/ap^{wow} females were determined when single pair mated to C(2L)VH2,lt;C(2R)P,px or to Basc/Y (standard second) males for four, one-day broods.

RESULTS

The estimated frequency of the four exceptional meiotic products from females that were rescued by sperm from compound-2 males was based on a multiplier system determined by the number of progeny. However, these determinations as well as comparisons among the experiments can be influenced by the following: 1) the frequency of egg hatch in the compound-2 strain that provided the experimental male parents, 2) the influence mating with genotypically (and chromosomally) different males had upon the rate of egg laying, and 3) the distribution and variance of progeny generated by the experimental females when crossed to standard males.

Generally it is assumed that compound autosomes assort randomly during meiosis in males, producing four classes of sperm in equal frequency. Moreover, aneuploidy for compound autosomes is known to result in embryonic lethality (Scriba 1967, 1969). It follows, therefore, that in crosses involving females whose compound autosomes show regular segregation the expected frequency of hatch (viable progeny) is 25 percent. Indeed for compound-3 strains, Holm and Chovnick (1975) found the percent hatch to differ insignificantly from that expected. However, for some compound-2 lines, slightly higher values have been obtained (Clark and Sobels 1973; Holm 1976). Since any estimate of the frequency with which aberrant meiotic products for chromosome 2 recovered from standard females is clearly dependent on the relative proportions of the four classes of sperm produced by compound-2 males, I tested the frequency of egg hatch for C(2L)VH2,lt;C(2R)P,px males crossed to C(2L)P,b;C(2R)P,px females. In a previous study of egg hatch frequency, approximately 25 percent was found in compound-2 crosses involving

males from the C(2L)P,b;C(2R)P,px strain (Holm, unpublished data). Similarly, as shown in Table I, there was no significant difference between the hatch I observed and the theoretical expectation of 25 percent. Therefore, I have concluded that the segregation of compound-2 chromosomes was random in those male lines used throughout this study.

The second consideration, namely the influence of different males on the rate of egg laying, was tested by crossing experimental females to males used in the multiplier system and males used to rescue aberrant meiotic products. It is evident, from the results recorded in Table II, that within this study the mean number of eggs laid was independent of the males used.

The use of a multiplier system enabled me to estimate the population size from which exceptional events arose. In this study the mean number of flies from each multiplier system varied greatly between experiments (Table III). In addition to differences in genetic background this variation was probably due to differences in such factors as the consistency of the medium (e.g. degree of hydration). As one precaution, therefore, all multiplier systems were run on the same batch of food as the corresponding experiment. A goodness of fit test confirmed that the number of progeny from the multiplier vials of each experiment were normally distributed. Although the mean number of progeny from each experiment differed, Bartlett's test for unequal sample size showed among the experiments there was homogeneity of variance. In view of the above findings, namely random segregation of compounds, normal distribution and homogeneity of variance, the values obtained from the multiplier system were taken as representing reasonable estimates of the mean number of progeny produced per experimental vial, and these values were used to calculate the frequencies of the various exceptional meiotic products. Moreover,

TABLE I

Percent hatch of eggs recovered from $C(2L)P,b;C(2R)P,px$ females mated to $C(2L)VH2,1t;C(2R)P,px$ males.

Number females tested	Total eggs laid	Total hatch	Percent hatch	95% C.I. *
35	2527	660	25.31	23.02 - 27.65

* The unweighted mean percent hatch and the 95% confidence interval were determined by using the arcsin transformation values of individual results.

TABLE II

Number of eggs laid by *In(2L + 2R)Cy/ap^{wow}* females when mated to *C(2L)VH2,1t;C(2R)P,px* or *Base/Y* (standard second) males.

Male	Brood				Total eggs laid	Number of females	Total mean number of eggs laid per female \pm S.E.
	1	2	3	4			
<i>C(2L)VH2,1t;C(2R)P,px</i>	1329	485	405	487	2706	41	66.0 \pm 2.15
<i>Base/Y;+/+</i>	1350	397	454	379	2580	40	64.5 \pm 2.00

TABLE III

Multiplier values obtained for and corresponding estimated total number of progeny expected in each of the experiments involving standard females crossed to compound-2 males.

Female genotype	Treatment in rads	Number of multiplier vials*	Mean number of progeny per multiplier vial \pm S.E.	Number of experimental vials**	Estimated total number of progeny
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	208	104.12 \pm 2.81	1945	203,000
<i>In(2L + 2R)Cy/lt pk cn</i>	0	105	63.85 \pm 1.57	874	55,800
<i>In(2LR)SML,Cy/ap^{wow}</i>	0	76	83.28 \pm 3.83	599	49,900
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	101	74.93 \pm 1.94	948	71,000
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	89	59.25 \pm 1.87	755	44,700
<i>In(2LR)SML,Cy/ap^{wow}</i>	2000	88	79.18 \pm 2.29	708	56,000
<i>+/ap^{wow}</i>	0	103	92.21 \pm 2.07	765	70,500
<i>+/lt pk cn</i>	0	88	71.18 \pm 2.01	880	62,600
<i>+/ap^{wow}</i>	2000	76	100.78 \pm 2.74	779	78,500
<i>+/lt pk cn</i>	2000	119	64.13 \pm 2.11	885	56,800

* Females crossed to *Basc* males.

** Females crossed to compound-2 males.

the random segregation of compound autosomes indicated that multiplying the measured value by four would provide a close estimate of the actual meiotic events.

Before examining the four aberrant classes and the frequency with which they were generated, it is important to briefly consider the effect of gamma radiation at high doses, i.e. above 1000 rads. In agreement with the observations made in this study on stage 14 oocytes, experiments show that stage 14 oocytes, the most mature stage, are extremely radio sensitive. It has been reported (Parker and Hammond 1957; Parker 1959) that at a dose of 2000 rads, approximately 97% dominant lethality can be expected at this stage. It should also be noted that Koch, Smith and King (1970), using the frequency of egg hatch as the measure of frequency of lethality, found at 2000 rads the relative lethality of stage 14 oocytes was 75% when corrected for control values. In the present experiment the period of time over which the females were collected (24 - 48 hours) did not guarantee the exclusion of all stage 14 oocytes. However, at the radiation exposure (of 2000 rads) used in these experiments most of the stage 14 oocytes should have been eliminated. In this series of experiments I was primarily interested in stage 7 oocytes as Parker (1969), and Parker and Williamson (1970) have shown that interchanges which occur at this time direct the segregation of the chromosomes involved which can result in nondisjunction. In contrast, segregation of stage 14 oocytes is already determined and is independent of radiation induced interchange (Busby 1971; Williamson 1973). One could expect sister strand compounds at this dosage (and one was recovered in a preliminary test) but non-sister compounds would be rare as the repair, it would appear, would be post-fertilization (Parker and Hammond 1958).

Although stage 7 oocytes were of prime consideration in this thesis, the magnitude of the number of virgins required (28,800 in 16 experimental crosses) in the recovery of the four classes of events made short collection times impractical and consequently a 48 hour collection period was used. I did, however, hold the virgins at 18°C on unyeasted medium prior to treatment. The literature dealing with dominant lethality of the two main stages, namely 7 and 14, is based on egg hatchability studies. I observed, from results which I will discuss in detail later, that a large proportion of the progeny from crosses between F(2L)b pr/In(2LR)SM1,Cy/F(2L)bw females and F(2L)nub b pr/F(2L)nub b pr;C(2R)r1 cn males, all of which were aneuploids, survived to pupation. Thus, at least for chromosome 2, lethality should be based on the frequency of viable progeny not the frequency of egg hatch. In order to determine the contribution of the more sensitive stage 14 oocytes to the recovered aberrant events I conducted the following experiments.

Approximately 1000 In(2L + 2R)Cy/Pin females were collected, aged a minimum of three days to ensure the presence of stage 14 oocytes, and treated with 2000 rads of gamma radiation without etherization. These females were single pair mated to compound-2 males in shell vials for 20 hours. Egg counts were done on approximately 100 of the vials. From these counts, and correcting for sterile vials, the total number of eggs laid by all females was estimated to be 16,500. The 19 recovered progeny from this experiment were as follows: 15 patroclinous (.091%), three matroclinous (.018%) and one sister-strand compound (.006%). (Percent recovery is based on the number of progeny recovered per eggs laid.) The mean number of progeny recovered per vial was .024 (19/796), therefore, in the experiment, using In(2L + 2R)Cy/ap^{wow} treated with 2000 rads (Table III), the estimated maximum contribution of stage 14 to the total recovered progeny based on the above would be 22.56

progeny (.024 x 948 vials). Correcting for this contribution the frequency of total aberrations (i.e. the sum of the four classes) would have gone from a percent frequency of .740 (Table V) to a corrected value of .708, a rather small decrease. Furthermore, 100 of the originally treated females were mated to males with normal chromosomes marked by b pr lt pk cn and brooded for the same 20 hours. Egg counts were performed on all vials and only those bearing fertilized eggs were included. Using eclosed progeny as the criterion, the survival rate of stage 14 oocytes treated with 2000 rads was calculated to be 5.96% (103 progeny/1,727 eggs). The mean number of eggs per vial was 18.37 with a standard deviation of 6.25. In addition, after 20 hours the parents were rebrooded for a period of 3.5 days, from which 5,906 progeny were recovered giving a mean of 59.06 progeny/vial with a standard deviation of 17.2. It should be noted that six of the females which did not lay eggs in the first 20 hours, did give progeny in the subsequent brood.

Finally, 150 structurally homozygous females collected and treated with 2000 rads within six hours of eclosion and aged two days following treatment, were mated to compound-2 males with 15 pairs per bottle and left for four days. A total of 37 progeny was recovered, of which 24 were patroclinous, five matroclinous and eight carried a newly induced C(2L) or C(2R). The proportion of the three classes to the total progeny recovered was 65% patroclinous, 13% matroclinous and 22% compounds which parallels the proportions recovered from structural homologues ($\frac{+}{ap}^{WOW}$) 63%, 12% and 24% respectively, as recorded in Table VI.

The above three findings using 2000 rads of gamma radiation, namely the low recovery of progeny from stage 14 oocytes (19 progeny/16,500 eggs), the low survival rate (5.96%) of stage 14 oocytes and the parallel proportions of

TABLE IV

Number of progeny recovered as exceptional chromosome-2 products of meiosis and the number of X-linked recessive lethals obtained in corresponding multiplier tests.

Female genotype	Treatment in rads	Estimated total number of progeny	Number of exceptional chromosome-2 products				Recessive X-linked lethals*
			Compounds (2L or 2R)	Matroclinous (Disomic-2 eggs)	Patroclinous (Nullo-2 eggs)	Equational Nondisjunctions	
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	203,000	0	137	220	6	13/1832
<i>In(2L + 2R)Cy/lt pk cn</i>	0	55,800	0	23	38	1	2/941
<i>In(2LR)SML,Cy/ap^{wow}</i>	0	49,900	0	34	41	4	4/950
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	71,000	38	159	328	4	27/857
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	44,700	21	89	138	1	14/821
<i>In(2LR)SML,Cy/ap^{wow}</i>	2000	56,000	10	92	169	7	18/715
<i>+/ap^{wow}</i>	0	70,500	0	10	9	1	1/981
<i>+/lt pk cn</i>	0	62,600	0	1	5	0	1/977
<i>+/ap^{wow}</i>	2000	78,500	85	43	223	3	13/727
<i>+/lt pk cn</i>	2000	56,800	33	30	78	2	13/958

* Number of X-linked lethals per total number of tested chromosomes obtained from the multiplier test. See text for full description.

TABLE V

Estimated percent recovery of the four classes of progeny that arose from aberrant chromosome-2 products of meiosis.

Female genotype	Treatment in rads	Estimated frequencies of aberrant chromosome-2 products (in percent)*				Percent recessive X-linked lethals**
		Compounds	Matroclinous	Patroclinous	Equationals	
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	.000	.067	.108	.003	0.71
<i>In(2L + 2R)Cy/lt pk cn</i>	0	.000	.041	.068	.002	0.21
<i>In(2LR)SM^L, Cy/ap^{wow}</i>	0	.000	.068	.082	.008	0.42
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	.054	.224	.462	.006	3.15
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	.047	.199	.309	.002	1.71
<i>In(2LR)SM^L, Cy/ap^{wow}</i>	2000	.018	.164	.302	.013	2.52
<i>+/ap^{wow}</i>	0	.000	.014	.013	.001	0.10
<i>+/lt pk cn</i>	0	.000	.002	.008	.000	0.10
<i>+/ap^{wow}</i>	2000	.108	.055	.284	.004	1.79
<i>+/lt pk cn</i>	2000	.058	.053	.137	.004	1.36

* Calculations were made using unmodified data.

** Based on results recorded in Table IV.

TABLE VI

Comparisons, within and between treatment groups, of the percentage each class contributes to the total number of exceptional progeny recovered from each experiment.

Female genotypes	Treatment in rads	Percent each class contributed to total aberrations				Homogeneity test**	
		Compounds	Matroclinous	Patroclinous	Equationals	χ^2	P
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	0.0	37.7	60.6	1.6	0.01	n.s.
<i>In(2L + 2R)Cy/l^t pk cn</i>	0	0.0	37.1	61.2	1.6		
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	*	32.4	66.8	0.8	3.27	n.s.
<i>In(2L + 2R)Cy/l^t pk cn</i>	2000		39.0	60.5	0.4		
Pooled Heterologues	0	0.0	37.6	60.7	1.6	3.77	n.s.
Pooled Heterologues	2000	*	34.5	64.8	0.7		
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	7.2	30.5	62.0	0.8	3.20	n.s.
<i>In(2L + 2R)Cy/l^t pk cn</i>	2000	8.4	35.7	55.4	0.4		
<i>+/ap^{wow}</i>	2000	24.0	12.2	62.9	0.9	6.99	n.s.
<i>+/l^t pk cn</i>	2000	23.2	21.1	54.9	1.4		
Pooled Heterologues	2000	7.6	31.9	59.9	0.6	93.09	<.05
Pooled Homologues	2000	24.0	14.6	60.3	1.0		

* Since no spontaneous compounds were recovered this class has been excluded to provide a direct comparison between treatment and controls for the percent distribution of the remaining three classes of exceptional meiotic products. Percent distributions including compounds are presented in lines 7 and 8.

** Based on actual numbers as recorded in Table IV.

each aberration between definite stage 7 oocytes and those ranging in age up to 48 hours support the concept of high radiosensitivity of the later stage. Even though a 48 hour collection period was used in the main experiments it would appear from these results that minimal contribution was made by any stage 14 oocytes which may have been present in the experimental females.

Frequencies Of Chromosomal Aberrations: The system confirmed that four classes of aberrancies are recoverable. There is a major trend to the results: radiation definitely increases the frequency of the recovery of disomic-2 eggs, nullosomic-2 eggs, and newly generated compound autosomes but does not measurably increase the recovery of equational nondisjunctions. Table V contains the estimated frequencies of each event based on the actual numbers recorded in Table IV. The experiments are grouped according to treatment and structural arrangement of the chromosomes involved.

While the frequency of recovering compound autosomes was higher for the structural homologues, there was a significant difference between the two lines of females studied (see Table VII). The +/lt pk cn heterozygote gave a lower frequency than +/ap^{wow}, which may have been due to the lower viability of sister-strand right arm attachments, as only one was recovered as compared to the recovery of eight compounds from the attachments of sister-strand left arms. Only one spontaneous compound was recovered and it was from a female heterozygous for ap^{wow}/In(2L + 2R)Cy. This compound is not recorded in the table because the experiment from which it was recovered had to be discontinued.

The matroclinous progeny are not significantly different within groups (see Table XII). However, between group comparisons reveal that the highest

frequency of matroclinous progeny was produced by females structurally heterozygous for chromosome 2. This was not expected and presented a direct conflict with the results of Day and Grell (1966) upon which I will comment later. One of the most important findings was the difference between the control and treated both for the structural heterologues and for the structural homologues, the increases being approximately 3X and 5X respectively.

Patroclinous progeny, in general, followed a pattern of recovery similar to that of the matroclinous class. However, the differences within groups were usually significant (see Table XIII). It would appear (as described in a later section of this thesis) that the frequency of the two events parallel one another, which may imply a common mechanism. However, the results suggest additional factors may have greater influence upon the formation of patroclinous than upon matroclinous progeny.

Moreover, the present study revealed for the first time the recovery of spontaneous equational nondisjunctions. In both induced and spontaneous the frequencies were estimated and while a comparison between spontaneous and induced usually showed an increase for the latter, the differences were found not to differ significantly.

Internal Dosimeter: Recessive lethal frequencies were determined for all experiments. As expected radiation treatment resulted in measurable increases in the recovery of recessive lethals. An unexpected observation, however, was the higher recovery of spontaneous X-linked recessive lethals from females structurally heterozygous for chromosome 2 as compared to those that carried structural homologues (Table V). While an earlier study (Thompson 1960) revealed that inversions on chromosome 2 and chromosome 3 would increase the frequency of spontaneous recessive lethals on the corresponding homologue,

both in males and in females, no increase in recessive lethals was shown for the X. However, studies on the X chromosome were conducted on males only.

DISCUSSION

Statistics: One of the main objectives of this research was to recover and quantify four classes of autosomal aberrations, three of which, based on evidence from earlier studies, were presumed to arise as products of chromatid interchange (for a review see Parker and Williamson 1976). Since the frequency of exceptional events had to be determined from an independent multiplier system this presented a statistical problem in that the numerator was from a Poisson distribution and the denominator was from an external normal distribution (both of these distributions were statistically confirmed). Statistics could not be found to determine the confidence interval of frequencies determined in this manner. Nevertheless, as I have shown, the sampling of the population for the multiplier system was large (approximately 10% of the total females used), the variance between experiments for the multiplier vials was homogeneous, the numbers of progeny recovered from the multiplier vials were normally distributed, and the number of eggs laid by the females was male independent. Therefore, the multiplier system appears to provide a good estimate of the population from which the aberrations were recovered. This was supported further by the reproducibility of frequencies of recovery of matroclinous progeny. Within any group, (defined by treatment and structural arrangement of the chromosomes) there was no significant difference (see Table XII). However, between any two groups, the differences were significant.

Assuming the validity of the multiplier system, there are few statistics which can cope with very low frequencies. For all statistical comparisons, among the recovered aberrant frequencies, I employed Stevens' Tables (Stevens 1942), using the correction factor for recoveries greater than 20.

Internal Analysis: Another approach to studying the frequency of recovery of chromosomal aberrations was to examine the results independently of the multiplier system. This was done by comparing between experiments the percentage each aberration contributed to the total aberrations recovered from that experiment (Table VI). This analysis indicates homogeneity for the controls of both the In(2L + 2R)Cy/ap^{WOW} and the In(2L + 2R)Cy/lt pk cn genotypes. Similarly, the results from both these lines when treated were also homogeneous. Because there was homogeneity within both experiments, the results within the group could be pooled and these pooled data could be compared between groups. Thus I compared the results of the structural heterologues between the control and the treated. The compound chromosome class was removed from the treated group as there was no equivalent class in the untreated group. From this it was shown the treated and untreated results were homogeneous. This would imply that radiation does not alter the types of aberrations but simply increases their frequency. With the exception of compound formation the mechanism for spontaneous or induced matroclinous or patroclinous progeny would appear to be similar. These results further indicate that the spontaneous aberrancies also arose primarily in stages prior to stage 14. It also should be noted that they arose randomly and never in clusters.

Moreover, the frequencies of exceptional events from the treated structurally homologous strains, +/ap^{WOW} and +/lt pk cn when compared, also proved to be homogeneous and thus could be pooled. However, when the pooled results of the treated structural heterologues are compared with the pooled results of the treated structural homologues it is evident that they are not homogeneous. Since the exceptional products recovered from the controls for the structural homologues were infrequent they did not lend themselves to

statistical analysis and therefore are excluded from Table VI.

These results clearly indicate that radiation increases the frequency of all aberrant events, excluding equational nondisjunction. However, changing the structural arrangement of the homologues significantly alters the proportions of the various exceptional classes, i.e. when structural homologues were compared to structural heterologues, compound chromosome formation was increased in the former and matroclinous progeny in the latter.

Internal Dosimeter: The internal dosimeter (i.e. recessive lethal test) provided an independent demonstration of the effects of radiation (Table V). While indeed this internal dosimeter is viewed as important here, it is even more important, as described in a later chapter, when chemical mutagens are employed. The recessive lethal test also suggests additional information concerning the use of the lt pk cn chromosome. Table V indicates that in every case where the lt pk cn chromosome was used a slightly lower, although usually insignificant, value was obtained for every aberrant class when compared with the results from experiments using the ap^{wow} chromosome. The recessive lethal test also indicates a lower value for the lt pk cn chromosome. This could imply that when irradiated the lt pk cn bearing flies did not receive as high a dosage of radiation. However, since it is true for all four experiments where the lt pk cn chromosome was irradiated this seems unlikely. One suggestion is the lt pk cn stock may have a lower sensitivity to ionizing radiation resulting in the production of aberrant events (Spieler 1963; Sankaranarayanan and Sobels 1976).

Chromosomal Aberrations: It can be seen from the results in Table V that structural heterozygosity leads to an increase in the recovery of matroclinous and patroclinous progeny while compound formation is decreased. However, no

measurable difference can be detected for the rather infrequent equational nondisjunction.

The possible mechanisms leading to these exceptional meiotic products of chromosome 2 are examined in depth in the following chapters and compared with a unifying hypothesis on the formation of these events (Parker 1969). Moreover, a genetic testing system has been developed which for the first time provides direct evidence that the hypothesis applies to the major autosomes.

Finally, the assay system was used to test two known chemical mutagens. From the classes of irregular progeny recovered, the possible modes of action of the mutagens were examined.

CHAPTER II

ANALYSIS OF RADIATION INDUCED COMPOUND CHROMOSOMES

INTRODUCTION

The development of compound autosomes represented a major contribution which furthered genetic analysis in *Drosophila*. They have made it possible to initiate a wide range of new inquiries as well as to extend to the autosomes experimental approaches that previously were restricted to the sex chromosomes: half tetrad analysis (Baldwin and Chovnick 1967), viability of sperm nullosomal for one or more of the arms of the major autosomes (McCloskey 1966; Lindsley and Grell 1969), spontaneous and induced nondisjunction and chromosome loss in females (Bateman 1968; Gavin and Holm 1972; Clark and Sobels 1973), gene conversion (Chovnick et al. 1970; Ballantyne and Chovnick 1971), genetic dissection and characterization of proximal heterochromatin (Hilliker and Holm 1975) and a genetic approach to the regulation of insect populations (Fitz-Earle, Holm and Suzuki 1973; Fitz-Earle 1976).

Compound autosomes are chromosomes composed of two homologous arms attached to a common centromere. Although they have been widely reported, little is actually known about the nature of their formation. The original compound autosomes in *Drosophila melanogaster* were constructed in the laboratory of E.B. Lewis (Rasmussen 1960). While at first they were referred to either as attached autosomes or as pseudo-isochromosomes (see Holm 1976) the term compound autosome was adopted following the conventional system of terminology proposed by Lindsley and Grell (1968). In contrast to the complex chromosome mechanics required for their initial formation, now that compound-2 and compound-3 chromosomes are available, additional compounds can be easily generated by treating females from a standard strain with ionizing radiation and mating them to compound-2 (or compound-3) males (Holm 1976). Moreover, they can just as readily be generated in males (Leigh and Sobels

1970).

In their experiments, Leigh and Sobels (1970) used males heterozygous for recessive markers on the second chromosome. The new compound autosomes they recovered were either heterozygous (called heteroisos) or homozygous (homoisos) for the paternal markers. The heteroisos were recovered from cells that were diploid at the time of treatment while homoisos were recovered both from primary and from secondary spermatocytes. Those from the latter obviously had to be sister-chromatid attachments.

Bateman (1968), upon mating radiation treated females bearing standard second chromosomes to males bearing compound-2 autosomes, recovered exceptional progeny, 25% of which were newly generated C(2L)'s and C(2R)'s (he referred to these as isochromosomes). By using proximal markers Bateman concluded that approximately 25% of the newly induced "isochromosomes" were homozygous at the centromere, suggesting they were sister-chromatid attachments. Holm et al. (1969) indicated that newly generated compounds homozygous for proximal markers derived from heterozygous standards, could also occur by a crossover proximal to the marker followed by a non-sister chromatid attachment. However, Gavin (1971) recovered new compound-2 chromosomes homozygous for proximal markers from females heterozygous for In(2LR)SM1,Cy, a balancer second chromosome that not only prevents crossing over but also by its physical structure cannot contribute to compound formation. This finding unquestionably demonstrated that true sister-strand attachments could be generated in females.

Although the generation of both sister and of non-sister compound-2 chromosomes in both sexes was confirmed, two fundamental questions remained

unanswered: 1) what was the nature of their formation, and 2) what was the frequency of their formation both as spontaneous and as radiation induced products?

For the mechanism of compound-autosome formation, Bateman (1968) proposed three models. His first model involved breaks on the same side of the centromere of two chromatids in which adjacent, rather than opposite, arms attached, resulting in a dicentric isochromosome and an acentric fragment. His second proposal involved "illegitimate" exchange formed by simultaneous breaks in two chromatids on opposite sides of the centromere with a subsequent rejoining of left arm to left arm or right arm to right arm in a manner similar to that proposed by Rasmussen (1960). His third proposal was similar to Darlington's (1939) in that it implied a modified version of the centromeric misdivision model.

While genetic recognition of compound chromosomes originated with Morgan's (1922) discovery of attached-X chromosomes in *Drosophila*, concepts on the nature of their formation have been highly influenced by the centromeric misdivision model originally proposed by Darlington (1939, 1940) to explain the occurrence of isochromosomes in plants. He observed, in cytological studies of *Fritellaria*, chromosomes which he called isochromosomes because he thought they were the centromeric attachments of sister chromatids. Darlington proposed that isochromosomes arose through a transverse division of the centromere in an univalent followed by the fusion of the half-centromeres of the sister chromatids. Consequently, as defined by Darlington, isochromosomes would be structurally isosequential from the point of attachment, that is they would neither bear duplications nor deficiencies, and would be isogenic.

Later Giles (1943) observed in a single plant of *Gasteria*, one chromosome that regularly gave rise to isochromosomes. In these studies isochromosomes appeared to arise from normally paired and oriented bivalents rather than from univalents. He suggested a possible precocious division of the centromeres followed by the fusion of two sister chromatids resulted in isochromosome formation. Therefore, while Giles supported Darlington's original concept he found that isochromosomes could arise from bivalents as well as univalents. (It is noteworthy that Bateman's third model corresponds closely to that of Giles.)

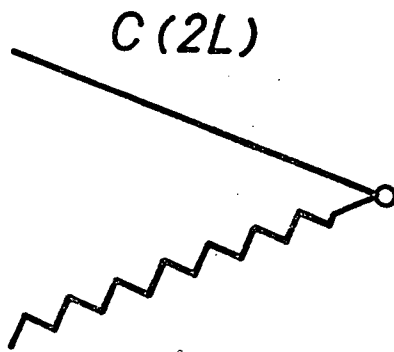
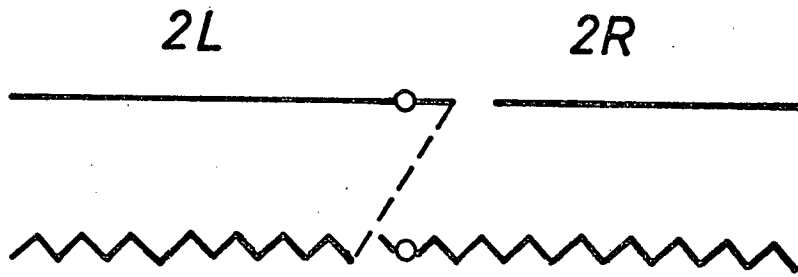
Brandam (1970) provided an alternative explanation to that of Giles (1943). He suggested that a single crossover within a pericentric inversion would result in apparent isochromosome formation. Brandam argues that they would not be true isochromosomes as they would only be isogenic distal to the point of crossing over. However, I wish to point out that this explanation would only account for non-sister chromatid attachments (pseudo-isochromosomes).

When she originally generated compound autosomes in *Drosophila*, Rasmussen (1960) favored independent breaks on either side of the centromere (called illegitimate exchange by Bateman). Leigh and Sobels (1970), studying compound-autosome formation in male *Drosophila*, also favored the model of two breaks on opposite sides of the centromeres resulting in newly formed compounds with small duplications and deficiencies (Figure 3). Observations leading to their support of this model were the following: 1) a high proportion of sterility was found among flies carrying a newly formed compound, 2) not all left and right compound combinations produced viable flies, and 3) many of the new compounds had very low viability.

Earlier evidence also supported a translocation-type event (or illegiti-

FIGURE 3

Compound chromosome formation is thought to arise from simultaneous breaks on either side of the centromere. The broken ends repair in such a way that the two left or right arms are attached to a common centromere.



mate exchange) giving rise to compound chromosome formation. Herskowitz, Schalet and del val Reuter (1962) studied induced crossing over in various regions of the X chromosome including the centromeric region as the females used were heterozygous for a euchromatic duplication (carrying the marker y⁺) on the right arm of one of the X chromosomes. From such females they recovered attached X's. Since compound-X formation is extremely rare in the absence of such a duplication, they suggested attachments were the result of pseudocrossovers with one break to the right of the centromere in a duplication-bearing chromatid, and the other to the left of the centromere in one of the other three chromatids. Such a mechanism would allow the formation both of sister and of non-sister attachments. Similarly, Neuhaus (1936) observed, in males, that attached X's could be generated only if the X chromosome carried a segment of the Y chromosome attached to the short arm.

Subsequent studies supported a similar type of mechanism for the autosomes. Kowalishyn (1971) generated compound chromosomes from a strain heterozygous for the SD-72 chromosome which carries a small pericentric inversion. When progeny carrying new right compound-2 chromosomes heterozygous for the SD-72 inversion were mated to compound stocks marked by the lt allele, the lt phenotype was suppressed. This demonstrated that duplications were tolerated as the new right arm carried the locus for the lt allele. This evidence supported the idea of the locus for lt being within the pericentric inversion and was later confirmed cytologically (Hilliker, personal communication).

In a subsequent investigation, Yeomans (1972) found in compound-2 strains generated from standard chromosomes, one right compound chromosome that, when in combination with a left compound homozygous for lt, suppressed the lt

phenotype. In view of this finding, all compounds, left and right, were tested for duplications of proximal loci on the opposite arms. Although no other rights were found to carry a duplication for the lt locus, approximately one-half of the left compounds carried duplications for the right proximal marker rl. These results indeed suggested that compound chromosome formation most likely was caused by breaks occurring on opposite sides of the centromere even as far out as the most proximal markers. Moreover, these findings supported a translocation-like event in which the broken ends rejoined attaching left or right arms to a common centromere in such a way as to generate compound chromosomes heterozygous for varying lengths of proximal deficiencies and proximal duplications of the opposite arms. In addition to radiation induced compound autosomes, one of the spontaneous C(2L) chromosomes recovered by Yeomans carried a duplication for the rl locus. This evidence suggested, therefore, that spontaneous, as well as induced, compound autosomes were the result of translocation-like events.

The objectives of the present research were two fold. The first was to confirm the translocation nature of compound autosome formation, in part, by determining whether new compounds carried duplications and, additionally by determining if the duplications included only those genes known to be located in heterochromatin (Hilliker and Holm 1975) or if they also included genes within the proximal euchromatin. The second purpose was to devise a relatively accurate system to determine the frequency of compound autosome formation in females.

The system for rescuing newly generated compound autosomes eliminated the recovery of all normal progeny. Thus, direct measures of frequencies could not be made. Leigh and Sobels (1970) attempted to quantify the fre-

quency of compound formation in males by the use of a multiplier system. The system involved mating males to females with standard chromosomes to provide an estimate of the population from which they arose. A similar approach was taken in this study and the arguments to support it have been given in Chapter I.

MATERIALS AND METHODS

Induction And Recovery: The genetic markers, mating protocol, treatment and recovery of compound autosomes were described in Chapter I.

The multiplier system, as previously described, provided the means of calculating the approximate frequency of compound-autosome formation. The frequencies were determined both for the structurally heterozygous and for the structurally homozygous combinations of second chromosomes.

Analysis Of Compound Autosomes Generated In Females Carrying The Genetic Marker ap^{WOW} : The use of the inverted chromosome, In(2L + 2R)Cy, which acts as an effective balancer for chromosome 2, in combination with a normal second proximally marked by ap^{WOW} on the right arm, allowed for distinguishing between sister versus non-sister chromatid attachments. All newly generated compound-2 left chromosomes were either phenotypically \pm , indicating a sister-strand attachment, or phenotypically Cy, representing a non-sister attachment, i.e. a compound-left autosome heterozygous for In(2L)Cy. This inversion carries in addition to the genetic marker Cy, recessive lethals which make it impossible to recover sister-strand compounds for the In(2L)Cy chromosome. Similarly, the right compounds could be classified as sister-strand attachments by the expression of the ap^{WOW} phenotype and the non-sisters by a \pm phenotype. Again the presence of recessive lethals associated with In(2R)Cy precluded the recovery of sister chromatid attachments of this chromosome. The genetic marker ap^{WOW} was used as it is viable when homozygous and in close proximity to the centric heterochromatin and thereby greatly reduced the probability of exchange occurring between it and the centromere, and event that would have led to the misclassification of compound-2R non-sister attachments.

In(2L + 2R)Cy/ap^{wow} (treated and control) females were mated to C(2L)VH2,lt;C(2R)P,px males. New compound-2L chromosomes were phenotypically identified as C(2L)+;C(2R)P,px (sister-chromatid attachments) or C(2L)Cy;C(2R)P,px (non-sister-chromatid attachments). Similarly, new compound-2R chromosomes were phenotypically C(2L)VH2,lt;C(2R)ap^{wow} (sister chromatid) or C(2L)VH2,lt;C(2R)+ (non-sister chromatids). The marker, lt, on the compound-2L chromosome from the males was selected specifically to provide immediate identification of newly induced compound-2R chromosomes that carried a duplication of proximal 2L including the lt⁺ allele. These flies were phenotypically wild type or phenotypically ap^{wow}. Furthermore, all progeny bearing newly generated left compounds were mated to C(2L)P,b;C(2R)VH5,r1 (b, black; r1, rolled) to identify new compound lefts carrying a duplication for the right arm proximal marker r1. Those that carried a r1⁺ duplication were crossed to C(2L)+;C(2R)stw (stw, straw) to determine whether the duplications extended distally as far as the stw locus, which is a proximal marker known to be located in euchromatin. [See Lindsley and Grell (1968) for the positioning of the above markers and see Hilliker and Holm (1975) for the relative position of lt and r1.]

One phenotypic class of progeny from this experiment, ap^{wow}, could have had three possible modes of formation. Flies that were phenotypically ap^{wow} could have been the result of the simultaneous recovery of left and right sister-strand attachments, thereby forming a disomic-2 egg rescued by a nullo-2 sperm. One such event was recovered previously (Gavin 1971). This phenotype could have also represented an equational or second division non-disjunction which would have been rescued by the same class of sperm. Additionally, as indicated above, this class could have represented a newly induced C(2R) sister-chromatid attachment carrying a duplication of 2L that

included the lt⁺ allele. Hence, to distinguish among all the three possibilities, progeny of this phenotype were mated in turn to flies of the following genotypes: C(2L)VH2,lt;C(2R)P,px and In(2L + 2R)Cy/bw^{v1}.

The structurally heterozygous experiments were repeated using the multiple break inversion In(2LR)SM1,Cy (Lindsley and Grell 1968). This balancer chromosome allowed for the recovery only of sister-strand left and right compounds from the chromosome carrying the ap^{wow} genetic marker. Since In(2LR)SM1,Cy carries a large pericentric inversion, compounds formed from this chromosome whether they were sister strand or non-sister strand attachments would produce inviable segmental aneuploids. New sister compound-2L chromosomes were phenotypically identified as C(2L)+;C(2R)P,px and sister compound-2R chromosomes were phenotypically identified as C(2L)lt;C(2R)ap^{wow}. As described above, all phenotypically ap^{wow} progeny were further tested to determine if they were equational nondisjunctions, simultaneous generation of left and right compounds or newly generated right compounds carrying a duplication of 2R including the lt⁺ allele.

In those experiments in which the second chromosomes were isosequential for the standard arrangement, but heterozygous for ap^{wow} (+/ap^{wow}), only the compound-2R's homozygous for ap^{wow} could be classified as sister-strand attachments. The +/ap^{wow} females were mated to C(2L)VH2,lt;C(2R)P,px males. New compound-2L chromosomes were phenotypically identified as C(2L)+;C(2R)P,px (both sister and non-sister attachments). New compound-2R chromosomes were phenotypically identified as C(2L)VH2,lt;C(2R)ap^{wow} (sister chromatids) or C(2R)VH2,lt;C(2R)+ (non-sister chromatids or sister chromatid attachments of the unmarked arm). Additionally, compounds recovered from +/ap^{wow} females were tested for duplications as described previously.

Analysis Of Compound Autosomes Generated In Females Carrying The Genetic

Markers lt pk cn: The above series of experiments were repeated excluding the combination with In(2LR)SM1,Cy, replacing the chromosome carrying the genetic marker ap^{wow} by a chromosome carrying the genetic markers lt pk cn. Females (treated and control) were mated to C(2L)P,b;C(2R)P,px males. From the structurally heterozygous females the new compound-2L sister strands were phenotypically lt and the compound-2L non-sister strands were phenotypically Cy (Cy sister strands are not recoverable for the reasons presented earlier). The new right compounds were identified as sisters by means of the pk cn phenotype and non-sisters were phenotypically ± (wild type).

Compound autosomes arising from sister-chromatid attachments in females structurally homozygous for chromosome 2 could be identified only for the left arm marked by lt or the right arm carrying the markers pk cn. Non-sister-chromatid attachments as well as sister-chromatid attachments for the unmarked chromosome were indistinguishable, i.e. they were phenotypically ±. Compound-2 chromosomes generated from experiments involving lt pk cn were not tested for duplications as the chromosome carried the lt marker.

RESULTS

Based on values estimated by the multiplier system, from all five control (0 rad) crosses recorded in Table IV, plus one additional cross involving In(2L + 2R)Cy/ap^{wow} females by C(2L)VH2,lt;C(2R)P,px males, which was not recorded in the table, only one spontaneous compound, a C(2L), was recovered from an estimated 500,000 progeny. While this exceptionally infrequent recovery of spontaneous events agrees with previous findings from this laboratory (Gavin 1971; Yeomans 1972), Chadov (1973) reported the recovery of 29 spontaneously generated compound-2 chromosomes. However, he failed to provide information pertaining to the magnitude of the experiments from which the chromosomes were recovered, and therefore no comparisons can be made.

In view of the rare recovery of spontaneous events it is evident that the vast majority of newly generated compound autosomes were radiation induced (Table VII). It is important to realize that, as a function of the meiotic behaviour of compound autosomes in males, only 25 percent of the newly generated chromosomes would be recovered. Therefore, the actual frequency of formation is approximately four times that measured, which in fact is strikingly close to the frequency of detachments of compounds at 2000 rads reported by Hilliker and Holm (1975).

In considering the mechanism of compound-chromosome formation one important parameter to measure is the frequency of recovering the various classes of expected attachment products. If, for example, compound autosomes arise as products of interarm interchanges (an interbrachial reciprocal translocation) C(2L) and C(2R) chromosomes would be expected in a 1:1 ratio. Moreover, since such events are viewed as interchromatid interchanges (Parker 1969,

TABLE VII

Estimated percent recovery of compound-2 chromosomes.

Female genotype	Treatment in rads	Estimated total number of progeny	Number of compounds (2L or 2R)	Frequency in percent	95% Confidence intervals*
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	203,000	0	0.000	0.000 - 0.002
<i>In(2L + 2R)Cy/lt pk cn</i>	0	55,800	0	0.000	0.000 - 0.007
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	71,000	38	0.054	0.038 - 0.073
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	44,700	21	0.047	0.030 - 0.072
<i>+/ap^{wow}</i>	0	70,500	0	0.000	0.000 - 0.005
<i>+/lt pk cn</i>	0	62,600	0	0.000	0.000 - 0.006
<i>+/ap^{wow}</i>	2000	78,500	85	0.108	0.083 - 0.133
<i>+/lt pk cn</i>	2000	56,800	33	0.058	0.040 - 0.081

* Based on the corrected fiducial limits of expectation from Stevens' Table (Stevens 1942).

Williamson 1969), sister and non-sister chromatid attachments both would be expected. The results for all four experiments in which females were treated with 2000 rads are recorded in Table VIII. Although the recovery of C(2L) and C(2R) are homogeneous in comparing all four experiments, the results do not fit the 1:1 ratio expected but rather a 4:3 ratio of compound-2L to compound-2R respectively. Even though each experiment is consistent in moving in the direction of a 4:3 ratio of C(2L) to C(2R), and the pooled results differ significantly from a 1:1 ratio, individual results do not depart significantly from the expected random recovery of the reciprocal products. These disproportions will be considered further in connection with the comparison between sister and non-sister chromatid attachments.

Regarding the involvement of the various combinations of chromatids, let us first consider the right-arm attachments of chromosome 2. In all experiments the right arm of chromosome 2 was heterozygous for a recessive marker, either ap^{wow} or pk cn. If, among the four chromatids, there was a random attachment of any two, there would be six possible combinations of any two chromatids involved in the formation of a new compound-right chromosome. Sister strand compound-2 formation would result from attaching two sister chromatids to the same centromere; non-sister formation would involve two chromatids, one from each homologue. In the present experiments the formation of a sister-strand compound could be detected by the recovery of C(2R) chromosomes homozygous either for the recessive marker ap^{wow} or for the markers pk cn. The recovery of such phenotypically marked flies, therefore, should be 1/6 of the total right compound-2 chromosomes produced from structural homologues. The results recorded in Table IX, for the structurally homozygous combinations, support the notion of random involvement of any two of the four chromatids, that is, 1/6 of newly induced right compound chromosomes were

TABLE VIII

Comparison of the recovery of compound-2 left vs. compound-2 right chromosomes.

Female genotype	Treatment in rads	Number of compound-left chromosomes	Number of compound-right chromosomes
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	24	14
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	12	9
<i>+/ap^{wow}</i>	2000	47	38
<i>+/lt pk cn</i>	2000	19	14
Totals		102	75 *

* The recovery of *C(2L)* to *C(2R)* was homogeneous ($P = .9$) for the nonrandom distribution of 4:3.

TABLE IX

Comparison of sister vs. non-sister compound-chromosome formation for the right arm of chromosome 2.

Female genotype	Number of sister- chromatid compound-2R	Number of non-sister- chromatid compound-2R
<i>In(2L + 2R)Cy/ap^{wow}</i>	3	11
<i>In(2L + 2R)Cy/lt pk cn</i>	1	8
<i>+/ap^{wow}</i>	6	32 *
<i>+/lt pk cn</i>	1	13 *
Totals	11	64

* This group includes sister-chromatid attachments of the unmarked (+) homologue.

homozygous for the recessive markers.

For the structurally heterozygous second chromosomes, theoretically 1/5 of the progeny should be homozygous for the recessive allele as the inversion on the right arm carries a recessive lethal which presents the recovery of sister-strand attachments for this chromosome and, in theory therefore, eliminates 1/6 of the potential compound-2R chromosomes. Again, Table IX shows that for structural heterozygotes the theoretical expectation was supported by the experimental results.

One of the difficulties in determining randomness of chromatids involved arises from the possibility of an apparent sister-strand attachment actually occurring as a result of compound formation from non-sister chromatids preceded by an exchange between the homologues. To reduce the possibility of exchange, proximal markers were used. The number of sister-strand attachments from structural homologues, where exchange could take place, is not significantly different from the number of sister-strand attachments from structural heterologues where exchanges are eliminated.

In addition, when the chromosome bearing the genetic marker ap^{WOW} was used in conjunction with the balancer, In(2LR)S^{M1},Cy, a chromosome bearing multiple inversions, crossing over is eliminated. The multiple rearrangements result in all non-sister and sisters from this chromosomes being inviable with the complementary compound from the male. The only recoverable compound chromosomes are sister-strand attachments from the ap^{WOW} chromosome, of which four rights were recovered.

Randomness for the left chromatids was also investigated where possible. For the chromosomes In(2L + 2R)Cy/lt pk cn randomness was observed (Table X).

TABLE X

Comparison of sister vs. non-sister compound chromosome formation for the left arm of chromosome 2.

Female genotype	Number of sister- chromatid compound 2L	Number of non-sister- chromatid compound 2R
<i>In(2L + 2R)Cy/αp^{wow}</i>	10 **	14
<i>In(2L + 2R)Cy/lt pk cn</i>	1	11
<i>+/αp^{wow} *</i>	-	-
<i>+/lt pk cn</i>	8 **	11 ***

* Sister-chromatid compound-lefts could not be identified.

** The recovery of sister-chromatid attachments greatly exceeded the expected ratios of 1:5 for cross 1 and 1:6 for cross 4. The recovery of sister-2L compounds fits a Poisson distribution.

*** This class includes both sister-strand attachments from the unmarked chromosome and the non-sister-strand attachments.

However, for the other two detectable cases, +1t pk cn and In(2L + 2R)Cy/ap^{wow} a higher proportion of sister-strand attachments than the model of randomness would predict, were recovered. These results are difficult to reconcile as in the latter case the increase cannot be attributed to crossing over. It is interesting to note that the recovery of left and right sister chromatids for In(2LR)Sml,Cy/ap^{wow} are approximately equal (six lefts, four rights), indicating that the excess recovery of sister lefts cannot be accounted for by the ap^{wow} chromosome, (data not included in Tables IX and X). The anomalous increase in recovery of sister lefts accounts for the greater total recovery of compound-2L's than compound-2R's.

The preferential recovery or formation of compound-2L is also found in the data of Bateman (1968) who recovered 430 lefts versus 392 rights and Chadov (1973) who recovered 28 lefts versus one right. These results indicate nonrandomness in the formation of compound-2 chromosomes, although no explanation can be given at present for this deviation from the expected.

From earlier work it has been demonstrated that it is extremely difficult to recover reciprocal translocations, especially in stage 7 oocytes (Traut 1967a, Parker 1969, Busby 1971). These results suggest that the formation of compound chromosomes in stage 7 oocytes would lead to the segregation of the reciprocal products of the translocation. This was supported by the finding that none of the putative equational nondisjunctions with the ap^{wow} chromosome proved to be reciprocal compounds. While the above findings are consistent with the concept that compound autosomes are products of reciprocal translocations of which only one-half the translocation is recovered owing to the interchange event in females, these results alone provide insufficient evidence to refute the model of centromeric misdivision. Evidence that the

event is indeed that of a translocation comes from the genetic analysis of newly generated compounds for duplications of genes located in the proximal regions of the opposite arm to that of the attachment.

Kowalishyn (1971) demonstrated through the use of a small pericentric inversion on the SD-72 chromosome that duplications and heterozygous deficiencies of proximal 2L and 2R heterochromatin could be tolerated. Moreover, Yeomans (1972) found that proximal duplications were sometimes produced as a result of compound formation. I tested all new compound chromosomes recovered from In(2L + 2R)Cy/ap^{wow} or +/ap^{wow} females for duplications of the complementary arm. The left compound chromosomes were tested for the proximal locus rl. As shown in Table XI, over 60% of the new left compounds, sister and non-sister strand attachments both, carried duplications for the rl locus. All those compound-2L chromosomes with the duplication for rl were tested further to determine if the duplication covered the more distal marker stw. However, none carried stw⁺. Similarly, newly generated compound right chromosomes were tested for a duplication of the left arm bearing the lt locus (Table XI). In one experiment (+/ap^{wow}) 13% carried the lt⁺ duplication. In the other experiment (In(2L + 2R)Cy/ap^{wow}) none were recovered. However, fewer compound chromosomes were generated in this experiment and the absence of duplications for lt was not statistically unexpected. In the cases for which C(2R) chromosomes carrying duplication lt⁺ were recovered, it was somewhat surprising that none were sister-strand attachments. Interestingly the single compound induced in stage 14 (see Chapter I) which was a sister-strand attachment for the right arm did carry a duplication for the lt locus.

TABLE XI

Genetic analysis of newly generated compound-2 chromosomes for the presence of duplications.

Female genotype	Compound-2 lefts carrying duplications of proximal 2R			
	$r\ell^-$	$r\ell^+$	total	percent with $r\ell^+$ duplication*
$In(2L + 2R)Cy/ap^{wow}$	6	10	16	62.5
$+/ap^{wow}$	14	25	39	64.1
Female genotype	Compound-2 rights carrying duplications of proximal 2L			
	lt^-	lt^+	total	percent with lt^+ duplication*
$In(2L + 2R)Cy/ap^{wow}$	13	0	13	0.0
$+/ap^{wow}$	33	5	38	13.2

* The results were homogeneous using G_H modified by applying Yates correction for continuity (Sokal and Rohlf 1969).

DISCUSSION

The present research provides a new approach for estimating the frequency of induced compound-autosome formation in females. The mechanism of compound-2 chromosome formation strongly favors the concept, originally proposed by Rasmussen (1960), of the joining of independent breaks on opposite sides of the centromere, a concept which Bateman (1968) referred to as illegitimate exchange. The evidence from this study strongly favors this proposal, i.e. radiation induces independent breaks on opposite sides of the centromere either of homologous or sister chromatids. To be recovered these breaks must rejoin in such a way that an acentric arm joins with an homologous (or sister) centric arm (Figure 3). The breaks occur randomly and rejoining can occur between any two of the four chromatids in the tetrad. Compound chromosomes generated in this manner would carry small duplications for the most proximal region of the opposite arm and would be heterozygous for proximal deficiencies. For example, a newly generated C(2L), as a consequence of joining an acentric 2L to a centric 2L chromatid, would carry a proximal segment of 2R and would be heterozygous deficient for a proximal segment of 2L.

Although never applied to the present situation, Parker (1969) used the term interchange to describe induced exchange between nonhomologues. Williamson (1969) extended the term to include interbrachial interchanges in the Y chromosome. In the present study chromatid breaks resulting in a new compound chromosome would be considered an interchange. Furthermore, Parker (1969) found that when interchanges took place between nonhomologues they tended to segregate at meiosis. In preliminary studies matroclinous progeny were tested to see if they were double compounds, but never was this the case. In the present study it was possible that a left and right sister-strand

attachment could occur simultaneously resulting in a fly phenotypically identical to an equational or second division nondisjunction of which several were recorded. All such flies were tested, but again none were found to be double compounds. Gavin (1971) observed one simultaneous recovery of a left and a right compound, both of which were sister-strand attachments. This product was recovered in a late brood and therefore must have occurred in an early stage of meiosis.

The strongest evidence for a translocation type event arose from a follow up of the original observations made by Kowalishyn (1971) and Yeomans (1972), that some newly generated compound autosomes carried duplications for proximal loci on the opposite arm. Upon testing all newly generated compounds recovered from In(2L + 2R)Cy/ap^{wow} or +/ap^{wow} females, I found 63% of the induced left arms carried a duplication that covered the marker rl located in the proximal heterochromatin of the right arm. Similarly, 9% of all right arms tested carried duplications for proximal regions of the left arm, which included the lt locus. Hilliker and Holm (1975) have demonstrated, through the detachments of compound seconds, that rl is located in the heterochromatin of the right arm of chromosome 2. Thus breaks resulting in the rl⁺ duplication occurred in the heterochromatic block between the rl locus and up to, or possibly including, proximal euchromatin. To test for breaks in euchromatin the compound left chromosomes were also tested for duplications involving breaks in the right arm distal to stw. None were found.

The failure to recover euchromatic duplications may be explained in three ways: 1) there is a low frequency of recovery of breaks distal to the euchromatic-heterochromatic junction, 2) flies are unable to tolerate autosomal duplications of this magnitude, and 3) the geometry of the chromosomal

arrangements at this stage of meiosis imposes a restrictive distribution on the interchange events such that heterochromatic breaks only rejoin with other heterochromatic breaks. It is interesting to note, in regard to the last point, that Hilliker and Holm (1975) found that, with the exception of one unusual three break product, all products of compound-2 detachments were restricted to heterochromatic-heterochromatic interchange events.

The recovery of over 60% of the breaks in the right arm distal to rl strongly implies that the rl locus falls approximately in the middle of the right heterochromatic block, supporting the conclusion derived from detachment studies (Hilliker and Holm 1975). Fewer lt⁺ duplications were recovered on new right compounds, possibly because it is more distal than rl. Again, this is supported by the detachment studies (Hilliker and Holm 1975). Many of the sister left compounds carried the rl⁺ duplication, indicating a common mechanism of formation both for sisters and for non-sisters. However, of the few rights carrying lt⁺ duplications none were sister-strand attachments.

As noted in the introduction to this chapter, results of other investigations also support a translocation like event. Neuhaus (1936) found that he could only recover spontaneous attached-X chromosomes in males if they carried an attached X.Y chromosome. The frequency was much higher when the attached X.Y involved Y^S rather than Y^L. He suggested, therefore, that a crossover between the Y fragment and the proximal region of the sister-chromatid X would result in the formation of an attached-X chromosome. Comparably, Morgan (1938) thought the origin of attached-X chromosomes was a two step crossover event. The first exchange between an X and a Y made the attached X.Y chromosome, and in a subsequent generation a second exchange between the attached X.Y and a normal X led to the formation of an attached-X

chromosome bearing a Y centromere. In both of these studies attached X.Y chromosomes were viewed as spontaneous events occurring in males.

Bateman (1968), although offering three models, appeared to favor the model of centromeric misdivision because the reversions he recovered from the detachment of compound-2 chromosomes were all homozygous viable. In contrast, Baldwin and Suzuki (1971), upon analyzing detachment products of compound-3 chromosomes, found that slightly less than half (66/162) were homozygous lethal. Similar results were found for the second chromosome (Hilliker and Holm 1975; Gibson, unpublished data). Although it has been realized that the inability to recover homozygous reversions is, in some instances, due to the presence of deficiencies in compound autosomes as a function of their formation (Baldwin and Suzuki 1971; Hilliker and Holm 1975), compound-2 chromosomes diploid for all genetic loci have been identified (Hilliker and Holm 1975).

While other models of compound chromosome formation cannot be excluded, they are not supported by the results obtained in this study. Darlington (1940) and Giles (1943) originally suggested centromeric misdivision as a method of compound chromosome, (or in their terminology isochromosome), formation in plants. Darlington's model, specifically, could only give rise to sister-strand chromatids and my results definitely show that compounds can be produced from both sister and non-sister chromatids. Bateman's (1968) model of centromeric misdivision incorporated the formation of both, but as previously mentioned, reversion studies indicated that deficiencies are produced in the formation of many compounds. Similarly, the formation of compound chromosomes leads to the recovery of duplications as supported by the findings in the present study.

Brandham (1970) thought that the isochromosomes (i.e. compound chromosomes) observed by Giles (1943) could have been the result of crossing over within a pericentric inversion. This model is plausible but fails to explain how sister-strand attachments are produced or how compounds are formed in male *Drosophila* where crossing over does not occur. The suggestion of Brandham is not supported by the findings of Kowalishyn (1971). He generated compound chromosomes from an SD-72/cn bw stock, where the SD-72 chromosome carries a pericentric inversion. The compounds induced were 19.8% of the total recovered progeny, whereas in the present study, with the +/ap stock, the induced compounds represented 23.8% of the total progeny. This would indicate that a pericentric inversion does not increase the formation of compound chromosomes and certainly is not a main contributing factor in fruit flies. Moreover, Gavin (1971) was able to generate compound chromosomes in the presence of the crossover suppressor, c(3)G, indicating that the normal mechanisms required for crossing over were not necessary for the generation of compound chromosomes.

Previously, the spontaneous recovery of compound chromosomes was reported by Gavin (1971), Yeomans (1972) and Chadov (1973). The frequency of such events I found to be extremely low, in that I recovered only one out of an expected 5×10^5 progeny. Nevertheless, this compound was of considerable interest in that it was formed through the attachment of sister chromatids of the left arm, and moreover, it carried a duplication for the r1 locus. This suggests that the mechanism for the spontaneous formation, either as sister or as non-sister attachments, is the same as that brought about by radiation. Finally, I conclude from the results of this study that compound autosomes arise as products of translocation.

CHAPTER III

ANALYSIS OF RADIATION INDUCED MATROCLINOUS AND PATROCLINOUS PROGENY

INTRODUCTION

The Sex Chromosomes: Early studies on radiation induced X-chromosome nondisjunction led to the recovery of matroclinous progeny as well as the reciprocal class, patroclinous progeny. However, the latter class was always more frequent (Mavor 1924). The increase in the frequency of patroclinous progeny above that of matroclinous progeny was defined as chromosome loss, an event thought to be separate and independent from nondisjunction, but recovered simultaneously.

Day and Grell (1966) examined the rate of X-ray induced primary nondisjunction with respect to structural heterozygosity. The sex chromosomes were appropriately marked so that the nondisjunctional female progeny (XXY) could be differentiated from the regular female progeny (XX), and the exceptional male progeny (XO) from the regular male progeny (XY). They used only the recovery of exceptional female progeny to determine the frequency of nondisjunctional events. Consequently this value had to be multiplied by a factor of four. Their study failed to show any significant difference in the frequency of induced nondisjunction of the X chromosome comparing structural homozygosity with structural heterozygosity. It is important to note that the homozygote used was homozygous for an inverted-X chromosome.

The type of chromosomal aberrations induced is dependent upon the maturity of the oocytes involved. Oocyte staging was carried out by King, Robinson and Smith (1956) and Koch, Smith and King (1970). The two main stages which can be readily identified are mature oocytes, which are considered to be in the latest stages of prophase I or early metaphase I, and classified as stage 14, and immature oocytes, which represent earlier stages

of prophase I and classified as stage 7. A particular stage can be treated by altering the time between eclosion of the flies and treatment. Those females treated immediately upon eclosion are considered to have their most mature oocytes at stage 7, while those flies that are aged for one day or more before treatment will have their most mature oocytes at stage 14. Traut (1970) reported the lack of stage sensitivity to radiation induced nondisjunction. This is in contrast to most other types of chromosomal aberrations for which mature oocytes show greater sensitivity (Parker and Hammond 1958; Parker 1959; Traut and Schmidt 1968; Traut 1967a, 1967b, 1968; Browning 1970; Traut and Scheid 1971).

As previously mentioned, Mavor (1924) concluded that the exceptional male class included products of nondisjunction as well as products of chromosome loss. To determine the frequency of chromosome loss, Day and Grell (1966) subtracted the number of matroclinous progeny from the total number of patroclinous progeny and multiplied by two, as only half of the patroclinous progeny could be recovered, i.e. the nullo gametes would only be recovered by X-bearing sperm. The exceptional males were genotypically X/O and resulted either from the reciprocal event of nondisjunction producing a disomic egg or through some radiation induced event leading to the loss of normally segregating X chromosomes. The excess of exceptional males was thought to be due to the latter event. Traut and Scheid (1971) have shown cytologically that chromosome loss may be partial or total in immature oocytes. This supports the earlier work of Grell, Munoz and Kirschbaum (1966) who found a correlation between the length of the chromosome and the induced loss.

Considerable evidence supports the idea of chromosome loss and nondisjunction being separate events although recovered in the same manner. Traut

(1971) demonstrated that dose protraction but not dose fractionation reduced the frequency of nondisjunction, while both reduce chromosome loss. Traut proposes that some chromosome loss arises from the fusion of two independent breaks in sister chromatids thus leading to dicentric bridge formation. This would agree with the reduced effect of protraction and fractionation as the first break would be repaired before a second break is produced. Chromosome loss as independent from nondisjunction is also supported by Muller (1940), Pontecorvo (1941) and Grell et al. (1966). Further evidence differentiating the two events comes from the study of Day and Grell (1966), who did find a stage sensitivity for chromosome loss, with the greater recovery in stage 14, but did not find stage sensitivity for nondisjunction. Hence it appears that there is general agreement that nondisjunction and chromosome loss represent two distinct and separate events.

The Autosomes: Since certain types of aneuploids for the sex chromosomes can be tolerated by *Drosophila*, namely XXY in females and XO in males, most of the studies on chromosome loss and nondisjunction have been limited to these chromosomes. However, Grell et al. (1966) introduced a new approach to studying these two phenomena by using a free-X duplication, which did not affect viability, together with a free fourth chromosome. The second fourth chromosome was present as a translocation with one of the major autosomes. The free fourth and the X duplication thus would enter a non-competitive distributive pairing pool and would regularly segregate, i.e. the X duplication would segregate from the free fourth (Grell 1962, 1964). When females of this genotype were treated with radiation the frequency of induced nondisjunction of the free fourth and the X duplication did not differ significantly from that observed for normal X chromosomes.

The first attempt at studying nondisjunction in the major autosomes was performed by Bateman (1968). He mated radiation treated females carrying standard chromosomes to males bearing C(2L);C(2R). This enabled the recovery of nondisjunctional products and loss of chromosome 2 as previously described in Chapter I. Ten percent of the total progeny recovered were matroclinous while sixty-six percent were patroclinous, the remainder carried newly formed compound autosomes. However, this approach enabled only relative values to be determined.

In a study similar to Bateman's, Evans (1971) made an attempt to quantify the events by examining eggs for hatchability frequencies. This type of experiment is severely limited in size and furthermore, demands a clear understanding of the developmental stages at which aneuploidy for chromosome-2 is lethal. Würgler, Ruch and Graf (1971) have also used the compound-2 system for studying induced chromosome loss in secondary oocytes. While they did reveal some chromosome loss at this stage, the test required the treatment of eggs 3 - 4 minutes after egg laying, and thereby severely limited the size of the experiment.

Gavin (1971) examined the effect of radiation on nondisjunction of the second chromosome in normal females mated to males carrying compound-2 autosomes. In her study no distinction was made between nondisjunction and chromosome loss. Gavin quantified this recovery of nondisjunction and newly formed compounds by crossing, in bottles, a number of treated females to normal males. This provided an estimate of the number of progeny that might be expected but was limited by the number of bottles used for the multiplier system and the effects of crowding from mass matings. It did demonstrate, however, a method of quantifying the exceptional events being studied. Gavin

and Holm (1972), again using multiplier bottles as an estimate of total progeny, measured the frequencies of nondisjunction and chromosome loss of the second chromosomes of irradiated females.

Clark and Sobels (1973) studied autosomal nondisjunction using irradiated females that were carrying compound seconds differentially marked from those carried by the males to which the females were mated. As mentioned previously, compound autosomes segregate at random in males, while in females compounds enter the distributive pairing pool (R.F. Grell 1962, E.H. Grell 1970, Holm and Chovnick 1975, Holm 1976). Provided the compound seconds are the only two chromosomes which enter the pool, they will normally segregate. Thus any induced nondisjunction resulting in disomic oocytes will be recovered by nullo sperm. The disomic sperm, as in previous studies, will rescue oocytes representing the reciprocal of the disomic egg class, which is chromosome loss. The normal segregational product in the female carrying either a compound-2L or a compound-2R will be viable with the complementary compound chromosome from the male. This allows the ratio of exceptional to normal events to be calculated in the same manner as in the X-chromosome studies. It should be noted that the attached arms in females pair for exchange in prophase I, and that the pairing does not influence the distributive pairing properties which are apparently responsible for their regular segregation. Therefore, caution must be exercised in attempting to compare the results from compound chromosomes to those of standard chromosomes.

Equational Nondisjunction: Although considerable effort has gone into the study of nondisjunction, attention has been focused primarily upon reductional nondisjunction, or nondisjunction of the first meiotic division. Nevertheless, from the work of Mavor (1924) we find that, although with con-

siderably lower frequencies, putative equational, or second division, nondisjunction also results from irradiation treatment of the X's. Gavin (1971), using heterozygous markers on the second chromosome, also reported autosomal equational nondisjunction both from males and from females. However, frequencies of these events were not determined. In the present study an attempt was made to identify and to determine the frequency of second-division nondisjunction of chromosome 2.

MATERIALS AND METHODS

The procedure and chromosomes used were as outlined in Chapter I. The matroclinous progeny for chromosome 2 were either phenotypically Cy (curly) or phenotypically + (wild type), depending on the females used. Patroclinous progeny were either lt px or b px depending on the compound-2 chromosome carried by the male.

Equational nondisjunctions were identified by the recovery of progeny homozygous for markers that were heterozygous in the female parent. In the case of ap^{wow}, a putative equational nondisjunction could have been the result of a crossover between ap^{wow} and the centromere followed by reductional non-disjunction. In such cases the progeny would carry the hypostatic marker Cy. All progeny that were putatively identified as arising from equational nondisjunctional events, from In(2L + 2R)Cy/ap^{wow} females, were tested for the presence of the Cy marker by mating them to + flies. The absence of the Cy phenotype in progeny from this cross was taken as confirmation of an equational nondisjunction. With the lt pk cn chromosome the recovery of progeny homozygous for markers lt and pk, which flank the centromere, was used to identify equational nondisjunctions. It is important to note that, in all experiments, products of equational nondisjunction could be identified only in those progeny bearing recessive genetic markers; products of equational nondisjunction of the unmarked chromosome could not be identified.

RESULTS

There are many ways of defining nondisjunction and chromosome loss. To reduce confusion and to avoid definitions implying possible mechanisms of formation, I will simply refer to the exceptional products either as matroclinous progeny, which clearly reflect reductional nondisjunction, or as patroclinous progeny, which may reflect nondisjunction or chromosome loss. The observed values of both the matroclinous and the patroclinous progeny will be described without any corrections as only relative frequencies are important. Estimated total frequencies of the actual meiotic occurrence can be obtained by multiplying the recorded values by a factor of four.

Spontaneous And Radiation Induced Matroclinous Progeny: The spontaneous recovery of progeny, matroclinous for the X chromosome, have been studied previously under three structural arrangements:

- 1) structurally normal homologues (Uchida 1962, Traut 1970),
- 2) structurally heterozygous homologues (Day and Grell 1966), and
- 3) inversion homozygotes (Day and Grell 1966).

In the present study, with the second chromosome, I considered only the first two configurations. The data in Table XII indicates that the structural organization of the chromosomes was more important in determining the spontaneous frequency of matroclinous progeny (both second chromosomes coming from the mother) than the specific genetic markers used on standard chromosomes. The spontaneous recovery of matroclinous progeny was not significantly different for the two inversions, In(2LR)SM1,Cy and In(2L + 2R)Cy, in combination with a normal chromosome. Similarly, the spontaneous frequency of nondisjunction did not differ significantly when comparing the structural homo-

TABLE XII

Estimated percent recovery of matroclinous progeny.

Female genotype	Treatment in rads	Estimated total number of progeny	Matroclinous progeny (Disomic-2 eggs)	Frequency in percent	95% Confidence intervals*
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	203,000	137	0.067	0.057 - 0.080
<i>In(2L + 2R)Cy/lt pk cn</i>	0	55,800	23	0.041	0.026 - 0.062
<i>In(2LR)SM^L,Cy/ap^{wow}</i>	0	49,900	34	0.068	0.047 - 0.095
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	71,000	159	0.224	0.191 - 0.262
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	44,700	89	0.199	0.156 - 0.245
<i>In(2LR)SM^L,Cy/ap^{wow}</i>	2000	56,000	92	0.164	0.133 - 0.202
<i>+/ap^{wow}</i>	0	70,500	10	0.014	0.007 - 0.026
<i>+/lt pk cn</i>	0	62,600	1	0.002	0.000 - 0.009
<i>+/ap^{wow}</i>	2000	78,500	43	0.055	0.040 - 0.074
<i>+/lt pk cn</i>	2000	56,800	30	0.053	0.036 - 0.075

* Based on the table or corrected fiducial limits of expectations from Stevens (1942).

zygotes, +/ap^{wow} and +/lt pk cn. However, there is a very significant difference in frequencies between the structural homologues and heterologues with the heterologues giving an increase of between five and twenty fold.

Contrary to my results, Day and Grell (1966) reported a higher recovery of matroclinous progeny from structural homozygotes, which were homozygous for an inversion, than from structural heterozygotes. They considered structurally inverted homologues to be analogous to structurally normal homologues. These values were presented as modified spontaneous values without reference to run size or confidence limits. I will consider this difference later.

The recovery of matroclinous progeny following radiation treatment (of 2000 rads) was three times that of the control with the In(2L + 2R)Cy/ap^{wow} chromosomes (Table XII). I repeated the experiment with a second balancer chromosome, In(2LR)SML,Cy, and again the radiation produced a greater than two fold increase in the recovery of matroclinous progeny.

I questioned the high frequency of induced abnormal meiotic behaviour of structural heterologues. In(2L + 2R)Cy involves a large inversion in each arm which greatly reduces crossing over (Ward 1923). The reduced crossing over between homologues would have increased the probability of their inclusion in the distributive pairing pool (Grell 1962). The spontaneous results suggested nondisjunction was related to the frequency of the entry of the chromosomes to the distributive pairing pool. This was in direct conflict with the results of Day and Grell (1966).

For the structurally heterozygous situation Day and Grell employed a normal X and an X with two inversions; In(1)d1-49 and In(1)Bar^{M1}. These two inversions reduce crossing over to twenty-four percent of that normally

observed (Cooper 1945). In the structurally homozygous condition both chromosomes carried the inversions. In this case crossing over was estimated to be ninety percent of the normal rate. The ratio of crossing over between structural heterologues and structural homologues is 24:90 giving a comparative crossover rate of approximately 1:4. However, they concluded the structural rearrangements did not lead to any significant difference in the frequencies of induced nondisjunction. The two structural combinations, as set up in my experiment, give a much higher ratio of crossing over. In the case of the +/ap^{wow} (structural homozygotes) there is an exchange between homologues about ninety-nine percent of the time and with the balancer, In(2L + 2R)Cy (structural heterozygotes), crossing over is reduced to less than one percent (Ward 1923). Therefore, the ratio of crossing over between the two combinations is 1:00 rather than 1:4. If I assume that those chromosomes which fail to undergo exchange pairing enter the distributive pairing pool, then the frequency of chromosomes entering this pool is inversely related to the crossover ratio. The results recorded in Table XII suggest that chromosomes which enter the distributive pairing pool are more sensitive to induced nondisjunction than are exchange chromosomes. Therefore, I would suggest that in order to detect the effect of structural heterozygosity on nondisjunction it is important to maximize the number of non-exchange chromosomes. My results show a four-fold increase in induced nondisjunction when the two chromosomes involved are structurally heterozygous rather than structurally homozygous. It is my impression that this increase reflects the greater availability of chromosomes to the distributive pairing pool.

This series of experiments was repeated with one modification, a standard chromosome marked by lt pk cn was substituted for the chromosome marked by ap^{wow}. As shown in Table XII this substitution did not lead to any signifi-

cant difference using the statistical method of Steven (1942). This lent support to the assertion that the events were real, and that the genetic background exerted minimal effect. Although there was no significant difference when the ap^{wow} chromosome was replaced by the lt pk cn chromosome, there was a generally consistent pattern of reduced values for the latter chromosome, implying there may be an underlying genetic influence in both the spontaneous and the induced experiments.

Spontaneous And Radiation Induced Patroclinous Progeny: The other major class of recovered progeny were patroclinous: progeny that received both compound-2 chromosomes from their father. In studies on the sex chromosomes, chromosome loss was usually defined as the difference between the number of matroclinous and patroclinous progeny. The patroclinous class was always recovered in a higher frequency and this was generally attributed to two events leading to their formation, namely nondisjunction and chromosome loss.

In the present study I define chromosome loss as the number of recovered patroclinous progeny, the reasons for this will be presented later. The frequencies of patroclinous progeny follow the same general pattern as nondisjunction (Table XIII).

As with matroclinous progeny, both radiation and structural heterozygosity had a marked effect on the recovery of the patroclinous class. Altering the structural homology increased the spontaneous recovery eight fold. Radiation increased the patroclinous class by a factor of four for the structural heterologues, which is similar to the observed increase in matroclinous progeny. The structural homologues showed approximately a 20 fold increase with radiation treatment over the controls.

TABLE XIII

Estimated percent recovery of patroclinous progeny.

Female genotype	Treatment in rads	Estimated total number of progeny	Patroclinous progeny (Nullo-2 eggs)	Frequency in percent	95% Confidence intervals*
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	203,000	220	0.108	0.095 - 0.124
<i>In(2L + 2R)Cy/lt pk cn</i>	0	55,800	38	0.068	0.048 - 0.094
<i>In(2LR)SML₁Cy/ap^{wow}</i>	0	49,900	41	0.082	0.059 - 0.112
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	71,000	328	0.462	0.413 - 0.515
<i>In(2L + 2R)Cy/lt pk cn</i>	2000	44,700	138	0.309	0.260 - 0.365
<i>In(2LR)SML₁Cy/ap^{wow}</i>	2000	56,000	169	0.302	0.258 - 0.351
<i>+/ap^{wow}</i>	0	70,500	9	0.013	0.006 - 0.024
<i>+/lt pk cn</i>	0	62,600	5	0.008	0.003 - 0.019
<i>+/ap^{wow}</i>	2000	78,500	223	0.284	0.248 - 0.324
<i>+/lt pk cn</i>	2000	56,800	78	0.137	0.109 - 0.172

* Based on the table or corrected fiducial limits of expectation from Stevens (1942).

The statistical analysis of the patroclinous progeny within groups showed greater variation than was shown for the matroclinous progeny. In the control for the heterologues, the ninety-five percent confidence limits overlap, but the means do not always fall within the limits. It would appear that genetic background plays a greater role in the recovery of patroclinous progeny than the recovery of matroclinous progeny. This confirms the earlier finding of Spieler (1963) who demonstrated higher spontaneous recovery of patroclinous progeny over matroclinous progeny and found both values varied with the strains used. The In(2L + 2R)Cy/ap^{wow} females gave a higher spontaneous patroclinous level than either In(2L + 2R)Cy/l^t pk cn or In(2LR)SM1,Cy/ap^{wow}. However, this was not the case for the matroclinous progeny. As indicated in Table XIII, significant differences for the recovery of patroclinous progeny exist among the different treated structural heterologues. Again, this was not shown for the recovery of matroclinous progeny. This difference, although significant, could be explained by the higher spontaneous rate for the In(2L + 2R)Cy/ap^{wow} females. However, statistics on data could not be corrected for spontaneous values as the determination of ninety-five percent confidence limits required the analysis be done on raw data, not on modified frequencies.

Equational Matroclinous Progeny: The third class of chromosomal aberration recovered arose from equational (or second division) nondisjunction. These progeny were matroclinous and homozygous either for the recessive marker ap^{wow} or for the markers lt pk cn. The frequencies for equational nondisjunction are presented in Table XIV.

The recovery of equational nondisjunctional products, from ap^{wow} heterozygous females, could have been the result of a proximal exchange

TABLE XIV

Estimated percent recovery of equational (second division) nondisjunctions.

Female genotype	Treatment in rads	Estimated total number of progeny	Number of equationals	Frequency in percent	95% Confidence intervals*
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	203,000	6	0.003	0.001 - 0.006
<i>In(2L + 2R)Cy/l^t pk cn</i>	0	55,800	1	0.002	0.000 - 0.009
<i>In(2LR)SML, Cy/ap^{wow}</i>	0	49,900	4	0.008	0.002 - 0.021
<i>In(2L + 2R)Cy/ap^{wow}</i>	2000	71,000	4	0.006	0.002 - 0.014
<i>In(2L + 2R)Cy/l^t pk cn</i>	2000	44,700	1	0.002	0.000 - 0.012
<i>In(2LR)SML, Cy/ap^{wow}</i>	2000	56,000	7	0.013	0.005 - 0.026
<i>+/ap^{wow}</i>	0	70,500	1	0.001	0.000 - 0.008
<i>+/l^t pk cn</i>	0	62,600	0	0.000	0.000 - 0.006
<i>+/ap^{wow}</i>	2000	78,500	3	0.004	0.001 - 0.011
<i>+/l^t pk cn</i>	2000	56,800	2	0.004	0.000 - 0.013

* Based on the fiducial limits of expectation (Stevens 1942).

between the marker and the centromere followed by reductional nondisjunction. The two events would have produced phenotypically identical progeny as the crossovers would carry the Cy marker, which is hypostatic to ap^{WOW}. To reduce the possibility of exchange, the ap^{WOW} mutant was used because of its proximal position to the centromere. All putative equational nondisjunctions, from In(2L + 2R)Cy/ap^{WOW} or from In(2LR)SML,Cy/ap^{WOW}, were mated to wild type flies to determine whether any carried the Cy gene. None, however, expressed this phenotype. Thus, all were classified as equational nondisjunctions. From the irradiated, In(2L + 2R)Cy/ap^{WOW} females, four progeny were phenotypically wild type, indicating a crossover had preceded nondisjunction. In this case, however, the exchange most probably occurred on the left arm between In(2L)Cy and the centromeres. None were found in the controls. This problem did not exist for the lt pk cn heterozygotes as the markers flank the centromere, therefore, the recovery of progeny homozygous for all three markers identified an equational nondisjunction. Table XIV shows that equational nondisjunctionals were recovered in all but one experiment. The frequencies were low and independent of the treatment or chromosomes used according to the ninety-five percent confidence intervals derived from Stevens' Tables.

DISCUSSION

Spontaneous And Radiation Induced Nondisjunction: Although the effect of heterozygosity for structural rearrangements on spontaneous and induced non-disjunction would appear not to have been previously intensively investigated, many experiments concerning induced nondisjunction of structural homologues have been recorded. From these studies have evolved a number of theories to explain the phenomena of induced nondisjunction.

Early concepts of the mechanisms of nondisjunction are reviewed by Lea (1955). Models have been proposed to explain the mechanisms of induced, but not spontaneous, nondisjunction. It is possible however, that in both cases the mechanisms may be similar. Some workers believed that radiation induced a physiological change in the chromosome which caused centromeres to adhere (Marquardt 1938; Carlson 1941; Sax 1941). These conclusions were derived from cytological observations of metaphase chromosomes since investigators thought they could see chromosomes adhering to one another. Darlington's (1942) explanation for chromosome stickiness suggested that the matrix of nucleic acid of a metaphase chromosome was in a polymerized non-sticky form. However, when irradiated, the matrix through a physiological change became more fluid and depolymerized resulting in surface stickiness. Marquardt (1938) suggested the change was reversible if the cells were in interphase at the time of irradiation.

Grell et al. (1966) argued that the centromeric region was the target because it was constant in size, and did not vary with the length of the chromosome. They based their idea on the observation that there was no significant difference between the nondisjunction of chromosomes of different

sizes, for example comparing X duplications and a free fourth with normal X chromosome. Day and Grell (1966) suggested that if chromosome stickiness were responsible for nondisjunction there would be stage sensitivity corresponding with the degree of condensation of the chromosome. Their results indicated that this was not the case because they found no stage sensitivity for nondisjunction during the first 12 days of egg laying. Similar findings have been reported by Traut (1970). Although Day and Grell's results suggested that a physiological change was not the cause of nondisjunction their data did not provide evidence for centromeric malfunction.

Day and Grell (1966) used a second method to study the nature of induced nondisjunction. They examined the effect of exchange and isosequentiality, and concluded that neither the absence of exchange nor the lack of isosequentiality affected the frequencies of radiation induced nondisjunction. However, I find that structural heterologues do cause a significantly increased rate of both induced and spontaneous nondisjunction and suggest that this may reflect the large differences in the frequency of exchanges that could take place between the two different combinations of homologues used in this study. My results, therefore, indicate that the rate of induced nondisjunction is affected by the frequency with which homologues enter the distributive pairing pool. The distributive pairing pool, as defined by Grell (1962), includes all chromosomes that failed to undergo exchange pairing. Moore and Grell (1971) found that various factors influence the association of chromosomes which enter the pool; the main one is that distributive pairing is size dependent but homology independent. The fourth chromosomes in female *Drosophila* rarely undergo exchange and, therefore, are usually members of the distributive pool. In my experiments involving In(2L + 2R)Cy/ap^{wow} as well as In(2LR)SML,Cy/ap^{wow}

both pairs of homologues were frequent members of the distributive pool. According to the size rule (Grell 1964), the difference in length between the fourths and the seconds would impede the association that leads to the segregation of these nonhomologues. The possibility of nonexchange X's, however, cannot be excluded.

One of the more recent models of nondisjunction has been proposed by Traut (1970). He found a threshold level for radiation induced nondisjunction of the X chromosome. The rate of nondisjunction was not influenced by fractionating the treatment. However, when the treatment was protracted, nondisjunction was reduced to one quarter. He suggested that the dependency of nondisjunction on both dose and dose rate made the target hypothesis of centromeric malfunction improbable; radiation may have disrupted one of the processes involved in spindle formation. This probably takes place in prophase (stage 7 oocytes). The effect of the irradiation would be a physiological change requiring a definite number of hits (multiple hit theory). This idea was supported by Forer (1966) who was able to interrupt chromosome movement during cell division by using an ultra violet microbeam to damage the spindle fibers.

In light of these models, my results concerning the recovery of matroclinous progeny, as well as the corresponding number of patroclinous progeny, might be explained as the result of chromosome stickiness, centromeric malfunction or spindle fiber damage. However, none of these models can explain the five fold increase in nondisjunction which occurs when the two chromosomes involved are structurally heterozygous.

To explain nondisjunction and chromosome loss, Parker (1969) proposed a

model that represents a distinct departure from those described above. His model stemmed from earlier work on the detachment of compound-X chromosomes in the presence of a free-Y chromosome (Parker 1954b). Detachments of the compound X involved translocations either with the Y chromosome or with the fourth in stage 7 oocytes. These detachments, he assumed, resulted from "induced-exchanges", which later he called interchanges (Parker 1969).

Parker (1969) concluded that two chromosomes formed a quasibivalent (e.g. the compound X and a fourth), which, owing to interchange, separated at meiosis I, while the recovery of the uninvolved fourth suggested random assortment. This supported his earlier findings. If the fourths always segregated owing to homology, but were not affected by interchanges, the free fourth recovered with the capped detached X would be either the sister chromatid of the fourth involved in the interchange or a chromatid from the free fourth homologue, and both would be recovered in equal frequencies. His results indicated that the chromatid from the homologue not involved in the interchange was recovered eighty-five percent of the time, while the sister chromatid of the involved homologue only ten percent. Consequently, Parker suggested that interchange usually led to the segregation of the fourth and the X, and that the production of eggs disomic for chromosome 4 were the result of the random movement of the non-involved fourth. In other words, interchange was an event leading to nondisjunction. This was tested further by Parker and Williamson (1970). When compound X females lacking a free Y chromosome were irradiated, the two predicted exceptional classes were recovered, i.e. the detachment product of a compound X and triplo-4 progeny. The latter class was found predominantly in males (269/278) as would be expected if the X usually segregated from the interchange fourth. Additionally,

the frequency of triplo-4 males, as well as the recovery of detachments of compound X's, increased in a nonlinear manner with dose, indicating that both events occurred as the result of double hits. They argued that, if chromosome stickiness or centromeric properties were responsible, nondisjunction of chromosome 4 would be independent of the segregational behaviour of the X chromosome. It should be noted, however, the argument applies only to immature stage 7 oocytes (Parker and Hammond 1957; Parker and McCrone 1958; Parker 1969; Parker and Williamson 1970).

The addition of a free Y to the female genome increased the recovery of triplo-4 progeny (Parker 1970). Evidently, four members in the distributive pairing pool not only increased the recovery of triplo-4 progeny, but also altered the sex-ratio recovery of triplo-4 progeny (4 males:1 female) as compared to the situation of no free Y where triplo-4's were recovered predominantly in males (30 males:1 female). In the absence of a Y chromosome, induced nondisjunction of the fourth would be primarily the result of interchanges with the X chromosome as described above. The presence of a Y chromosome, on the other hand, provided an alternative chromosome with which the fourths could undergo exchange. Consequently, it was proposed that such events led to the random movement of the X, which in turn sometimes produced eggs carrying an X chromosome as well as being disomic for chromosome 4. Thus, more disomic-4 female progeny were recovered from mothers with, than from mothers without, a free Y chromosome.

Parker and Busby (1973) extended the study of interchange mediated non-disjunction by using females with normal X chromosomes rather than compound-X chromosomes. As in the previous study, four chromosomes were involved. However, in this situation the two X chromosomes were independent, joined only by

chiasmata, and consequently not regular members of the distributive pairing pool. Parker and Busby (1973) suggest that in such a situation a quasitri-valent may form between the two X's and a fourth if an X-4 interchange occurs. In their results they estimate that seventy-five percent of all diplo-X eggs are nullo 4. Nondisjunction of the fourths occurred in oocytes in which the X chromosome had simultaneously been lost. Moreover, many of the triplo-4 exceptions showed structural alterations in one of the maternally derived fourths, in that a duplication for the tip of the X appended to the left arm of the fourth had been lost.

Interchange mediated nondisjunction of a pair of homologues, e.g. the X's, could involve reciprocal nondisjunction of another pair of homologues, e.g. the fourths. In such an event only one of the nondisjunctional products could be recovered. However, this was difficult to demonstrate with a structurally normal genome. Parker and Busby (1973) found that not all diplo-X gametes were nullo 4. Moreover, owing to the nature of their experiment, nullo-4 gametes could not be recovered. However, they did recover diplo-4 gametes that were nullo X. This implied that the reciprocal products, although not recoverable, were generated. Parker, Williamson and Gavin (1974) developed a system for rescuing the diplo-X;nullo-4 eggs by using males carrying a compound-4 chromosome. Therefore, the basic premise of their model was confirmed in normal genomes as one-half of the diplo-X eggs produced from irradiated females were nullo 4, while one-fourth of all nullo-X eggs were diplo 4. Furthermore, in those cases of nondisjunction, there was a higher incidence of structural alterations than in the disjunctional gametes. Thus the induced nondisjunction in normal genomes appeared to follow the same pattern as shown in previous studies involving modified genomes.

The assortment of interchange chromosomes in mature oocytes (stage 14) appeared to follow different segregational properties (Busby 1971; Williamson 1970a, 1974b). Busby found that C(1)-4 interchanges at this stage did not alter their normal segregational pattern. That is, both chromosomes involved in the interchange could be recovered at the same pole. This predicted that the recovery of reciprocal translocations in a single gamete was possible. Although none were actually observed, the conclusion was reached by observing that the sister chromatids of the fourths involved in interchanges were recovered with the detachment products in 15 out of 23 recoveries. This study indicated that when mature oocytes were treated, interchange chromosomes regularly disjoined from their homologues and consequently interchange did not lead to nondisjunction.

In the present research such events would be recovered infrequently as a radiation dose of 2000 rads eliminates most stage 14 oocytes if they are present (see Chapter I).

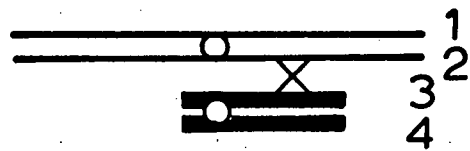
Quasibivalent Mediated Interchange Resulting In Autosomal Nondisjunction:

This explanation, when applied to my own findings, might be viewed in the following way: if the second chromosomes, owing to structural heterozygosity or the absence of exchange, are members of the distributive pairing pool, one of them, at the time of radiation, may have experienced an induced interchange with, for example, one of the fourths, thereby forming a quasibivalent (Figure 5). This interchange would direct the chromosomes forming the quasibivalent to opposite poles, while the remaining second and fourth chromosomes may (or may not) move at random. Parker (1969) reported that those chromosomes which did not go through interchange but were part of the distributive pool

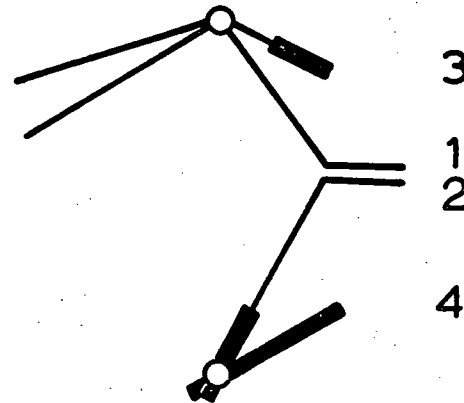
FIGURE 4

An example of an interchange forming a quasibivalent between a second and fourth chromosome.

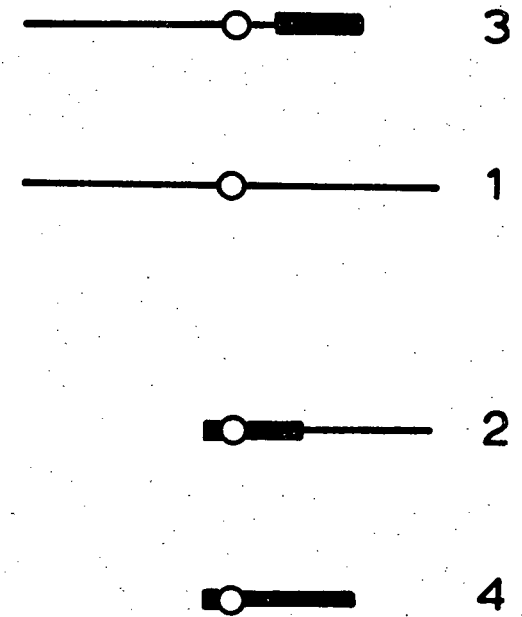
The interchange alters the length of the arms involved (2 and 3). Upon separation, the "drag" of the two longer chromatids (1 and 2) of each chromosome orients the centromeres, directing the shorter arms (3 and 4) to the poles of the cell. One of the polar or terminal chromatids (3 or 4) will be preferentially incorporated into the functional gamete. When mated to compound-2 males the nullo 2 (4) is rescued by a disomic-2 sperm whereas the free arm (3) is lost owing to aneuploidy.



INTERCHANGE



ANAPHASE I

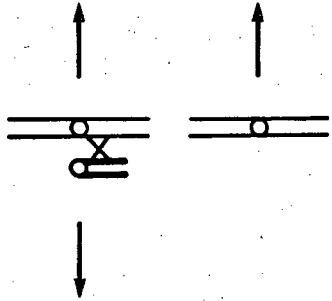






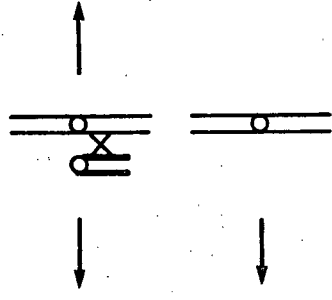

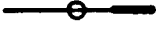
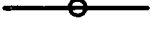
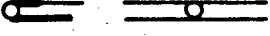




MEIOTIC PRODUCTS

FIGURE 5

An interchange between chromosome 2 and chromosome 4 produces a quasibivalent which causes the two heterologues to segregate at meiosis I.

The eight classes of gametes depicted represent those predicted from the formation of a quasibivalent.

MEIOSIS I	MEIOSIS II	GAMETES
 A	 A1	 A1 ¹ Capped 2nd
		 A1 ²
	 A2	 A2 ¹ Captured 2nd
		 A2 ²
 B	 B1	 B1 ¹ Capped 2nd
		 B1 ²
	 B2	 B2 ¹ Captured 2nd
		 B2 ²

tend to segregate from one another. In this manner then, during meiosis I, the two second chromosomes could go to the same pole, and a disomic or nullo-somic egg would be produced. While the involvement of chromosome 4 is assumed, it is possible that any other non-exchange chromosome could be involved in an interchange.

The above model provides a plausible explanation for the autosomal non-disjunction observed. It could also provide an explanation as to why structural heterologues yield a higher rate of nondisjunction than structural homologues. In the former case, the structural rearrangements would impede crossing over between the two second chromosomes, thus they would more frequently be members of the distributive pairing pool. Interchanges between one of the seconds and another chromosome, possibly the fourth, could then result in nondisjunction of the seconds. In this way, structural heterologues would enhance the recovery of nondisjunctional events.

Based on the above theory, the following predictions on autosomal non-disjunction can be made and tested. If an interchange took place between a second chromosome and a fourth there would be four possible meiotic products (Figure 4):

- 1) a normal chromosome 2,
- 2) a fourth with an attached-2 arm,
- 3) a chromosome 2 with a translocated arm of the fourth, and
- 4) a normal fourth.

In this manner, one of the chromatids of chromosome 2 would be broken into two parts, one bearing the centromere of the second, referred to as a capped second, and the other bearing the centromere of the fourth, referred to as a captured second. Based on the findings of Parker (1969) I will assume that

the non-interchange second and fourth chromosomes sometimes paired and segregated. The correctness of that assumption will not affect the basic premise of the model. In Figure 5-A the two second chromosomes could possibly segregate to the same pole in meiosis I, yielding the four classes of gametes as shown. When mated to compound-2 males, the only recoverable gametes would be the disomic two ($A1^2$), which would be rescued by a nullosomic sperm and the progeny would be phenotypically matroclinous, and the nullo-2 ($A2^2$), which would be rescued by a disomic sperm and the progeny would be phenotypically patroclinous. The other two gametes would be lethal with all possible sperm from compound-2 males. If the non-interchange second segregates to the opposite pole (Figure 5-B), the gametes would be as listed and all would be inviable with sperm from compound-2 males.

If the model applies to autosomal nondisjunction it predicts the formation of gametes $A1^1$ (Figure 5) which represents the left arm and centromere of chromosome 2 capped with an arm of the fourth, and $A2^1$ which represents the centromere of chromosome 4 with the right arm of chromosome-2. If the interchange takes place on the left arm, $A1^1$ will have a capped right arm and $A2^1$ a captured left arm.

Spontaneous and Induced Chromosome Loss: Generally it has been assumed that nondisjunction of any pair of homologues in females should result in an equal recovery of the reciprocal class, namely nullo gametes which when rescued by disomic sperm would be phenotypically patroclinous. Any excess in the latter class was thought to arise from causes other than nondisjunction and this excess generally has been referred to as chromosome loss. How can this excess of patroclinous progeny be explained?

The earlier work of Mavor (1924) originally showed that the induced recovery of matroclinous progeny was always less than that of patroclinous progeny; his control values did not reflect this difference. His study on the X chromosome was analogous to my study involving structurally homologous autosomes where I also recovered, from the untreated controls, both classes in equal frequencies, that is the number of matroclinous and patroclinous progeny were not significantly different (see Table IV). In contrast, the results from structural heterozygotes revealed not only a marked increase in the spontaneous level of nondisjunction but also a marked disproportionate recovery of the two classes with the patroclinous invariably the more frequent class. Similarly, the data of Sturtevant and Beadle (1936) with the X chromosome indicated that structural heterozygosity increased the recovery of patroclinous progeny relative to the recovery of matroclinous progeny. They suggested the higher recovery of patroclinous progeny could be explained by the occurrence of four-strand double exchanges within the inversion loop, resulting in the formation of two dicentric bridges. Consequently, the polar nuclei would not receive an X chromosome. Ptashne (1960) suggested that in structurally heterozygous X's, three strand double exchanges, one within the inversion and one between the centromere and the inversion could lead to an anaphase II bridge, resulting in nullo-X eggs. These models not only explain the present results but are supported by my observations on the balancer chromosomes used. In(2L + 2R)Cy which includes a large inversion both on the left and right arms of chromosome 2, could result in the formation of nullo-2 eggs via exchanges in a variety of ways. A four strand double exchange, one within each inversion loop, would lead to two dicentric bridges which might result in nullo-2 eggs. Similarly, a three strand double, one within the inversion and one proximal to the inversion, would form a dicentric at anaphase II which

might also result in nullo-2 eggs. Table XIII shows that the recovery of spontaneous patroclinous progeny was significantly higher than the recovery of matroclinous progeny (Table XII) for the structural heterologue, In(2L + 2R)Cy. On the other hand, the multiple-inverted chromosome, In(2LR)SM1,Cy, provided a far more effective balancer, which reduced crossing over, and consequently lowered the frequency of nullo-2 eggs. The data supported this as there was no measurable difference in the spontaneous recovery of matroclinous and patroclinous progeny from the experiment involving this chromosome.

Several explanations have been presented to explain induced X-chromosome loss. I will discuss these as related to my results with chromosome 2. The previous explanation of crossing over, which could explain spontaneous chromosome loss, could also account for induced chromosome loss as radiation increases the frequency of crossing over as specifically shown for chromosome 2 by Mavor and Svenson (1924) and Muller (1925). Induced crossing over is increased primarily in the heterochromatic region of chromosome 2 (Yeomans 1972) and this could lead to anaphase II dicentrics as proposed by Ptashne (1960).

Traut (1968) suggested that induced chromosome loss in stage 7 oocytes could result from the reunion of breaks in sister chromatids in such a way as to form acentric and dicentric chromosomes. An anaphase II bridge may result in nullo eggs. Previously, Traut (1964) observed that radiation induced chromosome loss did not follow linear kinetics. This implied that both one and two hit events were responsible for chromosome loss. However, this was later shown to be stage dependent. Traut (1968) examined chromosome loss both in stage 7 and stage 14 oocytes. He found that fractionation and protraction of the dose decreased the X-loss frequency with immature oocytes but had no

effect on mature oocytes. The linear kinetics obtained with mature oocytes suggested that single hits caused chromosome loss. In immature oocytes the effects of protraction and fractionation suggested a two hit event. Traut postulated that in stage 14 oocytes the chromatids were broken and the lack of a repair mechanism led to eggs with terminally deleted chromosomes. Consequently, during the first division of the zygote a bridge had been formed resulting in the loss of the corresponding chromosome.

Day and Grell (1966) looked at the effect of isosequentiality on the frequency of induced chromosome loss. They found no significant difference, and this, as in the case of nondisjunction, may have been due to the small difference in the frequency of crossovers between the two structural configurations they examined. In the present study there was a direct correlation between chromosome loss and the inability of the chromosomes to engage in exchange.

The investigations of interchange mediated nondisjunction led to the conclusion that the nondisjunction of one pair of homologues was not independent of that of another pair of homologues; one chromatid from each of two heterologues was involved in the interchange (Parker 1970). Similarly, using cytological techniques, Traut and Scheid (1971) recorded that eighteen percent of triplo-4 progeny were X0 (complete X loss), which is in general agreement with genetic analysis of Parker and Busby (1973) who found twenty-two percent of diplo-4 gametes were nullo X. The data from Parker and Busby (1973) indicated that the probability of recovering nondisjunction of the fourth chromosomes in a nullo-X gamete, rather than in a gamete with a single X, was two orders of magnitude greater. Their explanation suggests that a common event, an interchange between an X and a fourth, could lead to both

results, namely the loss of an X and the gain of a fourth.

The interchange between heterologous chromatids could result in both non-disjunction and chromosome loss, one being the reciprocal of the other. However, the equal recovery of matroclinous and patroclinous classes, as predicted for reciprocal events, was not realized. An explanation is required for the excess of the latter case.

The work of Novitski (1951, 1967) demonstrated that interchange, which results in asymmetrical dyads, does not result in randomness of recovery. His observations were based on exchanges between two X chromosome inversions, $\text{In}(1)\text{sc}^4\text{sc}^8$ and $\text{In}(1)\text{sc}^8$. The proximal breaks of these inversions are heterochromatic, $\text{In}(1)\text{sc}^8$, however, is longer than $\text{In}(1)\text{sc}^4\text{sc}^8$. An exchange between the two inversions results in a heteromorphic dyad of unequal lengths. The shorter exchange product bearing the tip of $\text{In}(1)\text{sc}^4\text{sc}^8$ is recovered more often than the longer chromatid bearing the tip of $\text{In}(1)\text{sc}^8$. In the case where the dyads involved are of different length, the segregation is dependent upon the orientation imparted upon the centromere. The two longest chromatids will be the last to separate at the metaphase plate. This "drag effect" of the long chromatids will orient the centromeres of the long chromatids so they are innermost in relation to the shorter two chromatids (Figure 4). In Drosophila females, the four products of meiosis are arranged linearly with one of the two outermost becoming the egg pronucleus (Sturtevant and Beadle 1936, Sonnenblick 1965, also see review by King 1970).

Subsequent studies have supported the general premise that the shorter chromatid will be recovered non-randomly. Parker and McCrone (1958), in detaching the C(X) chromosome recovered 21 Y-chromosome attachments carrying the y^+ duplication, while they recovered only four of the complementary class of

XY^LY^S. In those detachments involving only one arm of the Y chromosome, the short arm was recovered more frequently than the long arm (19:9). Parker and Williamson (1970) found that nullo-X;diplo-4 progeny were more common than C(X), nullo 4. This would support the recovery, following an interchange event, of the shorter chromatid as the fourth chromosome is much shorter than C(X). Finally, Parker (1974) using an X·4R translocation was able to generate both compound-X and compound-4R chromosomes. Theoretically, they should have been produced in equal numbers, providing both types of compound chromosomes were recovered randomly. The results, however, supported the concept of preferential recovery of the shortest arm of the interchange chromosome as Parker recovered 52 compound-4 chromosomes and only ten compound-X chromosomes. The compound fourths would have been formed from the shorter chromatid of the heteromorphic dyad produced by an interchange and thus would have had a greater probability of being incorporated into the polar nucleus.

In summary, the formation of gametes nullosomic for a given chromosome has been interpreted in several ways: 1) they could be the reciprocal event of nondisjunction, and this event may be the result of an interchange between nonhomologues (Parker 1970; Traut and Scheid 1971; Parker and Busby 1973; Parker, Williamson and Gavin 1974); 2) they could arise from four-strand double crossovers which would result in the formation of double dicentric bridges at meiosis I (Sturtevant and Beadle 1936) or from three-strand doubles which would result in single dicentrics at meiosis II (Ptashne 1960), both of which are supported by my results; 3) finally, they may be formed by the re-union of sister chromatid breaks forming a dicentric bridge at meiosis II (Traut 1968). The second and third interpretations must be considered with reservation as Novitski (1955) demonstrated that not all anaphase bridges give

rise to chromosome loss.

Proposed Mechanism For The Induction Of Chromosome 2 Loss: In the present study crossing over may have contributed to both spontaneous and induced recovery of patroclinous progeny. Induced crossing over from radiation treatment, specifically within inversion loops, cannot account for the four-fold increase in the frequency of recovery of patroclinous progeny as radiation does not appear to increase crossovers in euchromatic regions to this extent (Roberts 1962; Yeomans 1972). Therefore, interchange may be the largest contributing factor to the recovery of the patroclinous class. The interchange model applied to the present study suggests that the second chromosomes occasionally pair with a nonhomologue, possibly one of the fourth chromosomes. Should an interchange occur, it would direct the segregation of the second and the involved (fourth) chromosome to opposite poles. If, in addition, the "drag hypothesis", as suggested by Novitski, is true, the shorter chromatids at anaphase II would more frequently be polar and consequently would be included in the egg pronucleus (Figure 4). This would include the uninvolved sister chromatid of the fourth chromosome that engaged in the interchange and the second chromosome interchange chromatid that was capped by the fourth (i.e. bearing the centromere of chromosome 2). If I assume that the other second and fourth distributively pair and orient at random, with respect to the interchange pair, then in half of the events the normal second will go to the same pole as the capped second (Figure 5 A1¹). The capped second along with the normal homologue will produce an inviable segmental aneuploid with any sperm from compound 2 males. Consequently, the gamete is lost. However, as mentioned by Novitski (1967) the longer chromatids (in this study the normal seconds) will not always be excluded from the

polar nucleus. When it is included, and the uninvolved second goes to the same pole, a nondisjunctional event will arise and be recovered as a matroclinous progeny (Figure 5 A1²). As a consequence of interchange, the opposite pole, as shown in Figure 5, would most frequently receive the normal fourth and no second resulting in patroclinous progeny receiving both compound-2 chromosomes from their father. From this it may be suggested that nondisjunction and chromosome loss of autosomes are interrelated events arising as a consequence of nonhomologous interchange. The former has a lower recovery rate than the latter owing to the drag effect. It is also possible that the second and fourth chromosomes not involved in the interchange move at random with respect to one another (i.e. they do not distributively pair). If this were so, not all nondisjunctions of the second would be maternally nullo for chromosome 4. Parker and Busby (1973) and Parker, Williamson and Gavin (1974) suggest that with two free X's in the female there may be quasitivalent formation, assuming chiasmata formed between the X's with only one free member. However, the large number of variable combinations makes this difficult to examine.

Summary Of Nondisjunction And Chromosome Loss: In summary, these two phenomena, namely induced nondisjunction and chromosome loss of the second chromosome, may both be mediated by an interchange event. The formation of a quasibivalent, leading to an interchange, would direct one of the second chromosomes and the other chromosome involved in the interchange to opposite poles. The uninvolved second may segregate at random in which case, some of the time, the result would be the inclusion of both second chromosomes into the egg nucleus. Such a nondisjunctional event would be recovered as a matroclinous progeny.

On the other hand, the "drag hypothesis" predicts the preferential recovery of the shorter chromatids from the interchange chromosomes. Assuming chromosome 4 is the other interchange chromosome, it would be more frequently included in the egg nucleus. Thus, what is classically referred to as chromosome loss may be a reflection of the more frequent recovery of specific chromatids from an interchange event.

The above model explains not only both events but also the high frequency of loss versus nondisjunction. Moreover, the lower recovery of matroclinous progeny may result, in part, from diplo-2 gametes being nullo 4. Haplo-4 progeny from such gametes would only have an eighty percent viability (Lindsley and Grell 1968), which could make a significant contribution to the decrease in the recovery of the matroclinous class.

Such an explanation to account for nondisjunction and chromosome loss, apart from its intrinsic appeal, provides a unifying explanation of the events leading to the formation of disomic and nullosomic eggs. More importantly, the model can be tested by developing an appropriate selective system to recover the interchange products, namely the capped or captured single arms of an autosome.

Equational Nondisjunction: In all but one of the experiments, equational nondisjunctions were recovered, although in very low frequencies (Table V). Clearly such events have been shown for the X chromosome in males (Neuhaus 1936, Morgan 1938, Zimmering 1962). However, other than those studies designed to examine primary nondisjunction of the X's, no conclusive evidence has been documented for equational nondisjunction in females (see Zimmering 1976).

The results in this study clearly demonstrates the occurrence of

equational nondisjunction of autosomes. This is substantiated by two methods: the use of the balancer, In(2LR)SML,Cy, which produces lethal aneuploids through any exchange and the use of markers that flank the centromere. It is also interesting to find that equational nondisjunctions do not appear to be affected by radiation, structural homology or the chromosome used.

CHAPTER IV

AUTOSOMAL FREE-ARM FORMATION
AS SUPPORT OF A UNIFYING THEORY
FOR AUTOSOMAL PRODUCTS OF
MEIOSIS ARISING FROM RADIATION
INDUCED INTERCHANGES

INTRODUCTION

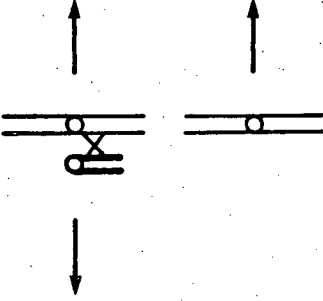
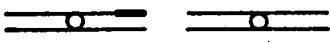





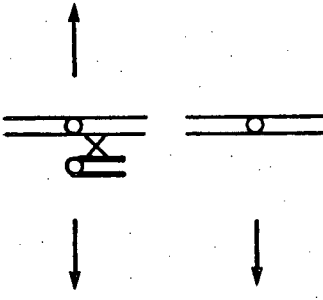
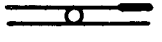
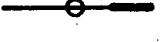
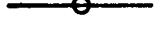
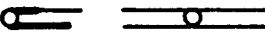


In the previous chapter I suggested that nondisjunction of chromosome 2 could be the result of an interchange with another chromosome in the genome, thereby forming a quasibivalent. The interchange would direct chromosome 2 and the other chromosome involved in the quasibivalent to opposite poles at meiosis I (see review by Parker and Williamson 1976). If the uninvolved homologue assorted at random, it may, in a certain proportion of the events, also move to the same pole as the involved chromosome 2. If, during meiosis II, a chromatid from the uninvolved second chromosome went to the same pole as the non-interchange chromatid from the dyad involved in the interchange (Figure 6, A1²) the resulting egg would be disomic for chromosome 2 and recovered as a matroclinous progeny. The reciprocal class, the nullo-2 eggs, would be expected in equal numbers. However, in all experiments involving radiation treatment, the recovery of nullo-2 eggs greatly exceeded the recovery of disomic-2 eggs. A large contribution to the excess recovery of nullosomic-2 eggs may have derived from quasibivalents because of the shorter chromatids being oriented in such a way that they were directed into the polar egg pronucleus. Such a segregation would have resulted in the polar nuclei being as shown in A1¹ and A2² or B1¹ and B2² of Figure 6. However, as indicated in the discussion of Chapter III other factors may have contributed to the excess recovery of the nullosomic class.

The quasibivalent model not only accounts for the greater recovery of patroclinous progeny over matroclinous progeny, but also it provides an explanation for the dramatic increase of both classes when the two second chromosomes are structurally heterozygous rather than structurally homozygous.

FIGURE 6

An interchange between chromosome 2 and chromosome 4 produces a quasibivalent which causes the two heterologues to segregate at meiosis I.

The eight classes of gametes depicted represent those predicted from the formation of a quasibivalent.

MEIOSIS I	MEIOSIS II	GAMETES
 A	 A1	 A1 ¹ Capped 2nd
		 A1 ²
	 A2	 A2 ¹ Captured 2nd
		 A2 ²
 B	 B1	 B1 ¹ Capped 2nd
		 B1 ²
	 B2	 B2 ¹ Captured 2nd
		 B2 ²

Inversions interfere with normal exchange and therefore increase the probability of chromosomes segregating as a function of the distributive pairing phase of meiosis (Grell 1962). In the distributive phase (which is also referred to as the distributive pairing pool) an interchange could occur either between homologues or any other member in the pool. Induced exchange between heterologues would direct their segregation which, in turn, could result in the formation of disomic or nullosomic gametes. I hesitate to suggest that only nonexchange chromosomes are involved in interchanges leading to nondisjunction for there is no experimental evidence to support this. Nevertheless, it is of interest to find that the greater frequency of induced exceptional events occur in females structurally heterozygous for chromosome 2.

Based on this model several predictions can be made. When the treated females are mated to compound males only gametes $A1^2$ and $A2^2$ (Figure 6) can be recovered. The $A1^1$ and $A2^1$ gametes are lost owing to the inviability of segmental aneuploids. The first prediction of the model is the formation of free left arms and free right arms of chromosome 2 as shown in Figure 6. The second prediction is based on Novitski's (1951, 1967) "drag hypothesis" which predicts the more frequent recovery of the shorter chromatid, a prediction supported by the work of Parker and McCrone 1958; Parker and Williamson 1970; and Parker 1974. The shorter chromatid recovered from an interchange complex can be identified by determining the origin of its centromere. Based on the above hypothesis, and assuming the involvement of the fourths, of the free arms recovered, more should be capped (have the centromere of the second, $A1^1$ Figure 6) than captured (have the centromere of the other chromosome involved in the quasibivalent, $A2^1$ Figure 6) (Parker 1969; Parker and Busby 1972). This is not to suggest that all left arms would be capped nor conversely that

all right arms would be captured. If the interchange were to take place on the left arm rather than the right arm of chromosome 2, as shown in Figure 6, the reverse results would occur (i.e. left arms would be captured and right arms would be capped).

Assuming the uninvolved second moves at random, the model predicts that some of the free arms should be recovered with their homologue, thereby suggesting that nondisjunction takes place as a result of interchange (Figure 6 A1¹). Moreover, if the uninvolved second moves at random, the model predicts the equal recovery of left and right free arms.

The recovery of the individual left and right free arms would give support to the theory of interchange as being a mode of generating progeny that are matroclinous or patroclinous for a major autosome. The generation of C(2L);F(2R) stocks by Holm (unpublished data) following the system developed by Grell (1970) enabled the above prediction to be tested. Free-2R stocks consist of a compound-2 left chromosome, as previously described and two free right arms (Figure 7). In males the homologous free arms normally segregate from one another and the compound-2L evidently moves at random. Gametes of two classes are produced: those bearing the compound-2L and a free-2R and those with only a free-2R (Figure 7). These males allow for the recovery of free arms generated through interchange as predicted by the above model. Interchange product A1¹ (Figure 6) would be rescued by a sperm carrying a right free arm and interchange product A2¹ (Figure 6) would be rescued by a sperm carrying a compound-2L plus a free-2R as demonstrated in Figure 8.

Such a system for the recovery of interchange-generated free arms of chromosome 2 made it possible to test the various predictions presented above.

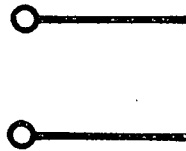
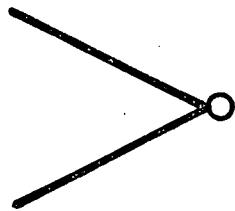
FIGURE 7

The segregation of free-2*R* chromosomes is shown for males. The two free-2*R* chromosomes segregate while the compound chromosome moves at random.

SPERMATOCYTE

C (2L)

F (2R)



GAMETES



A

B

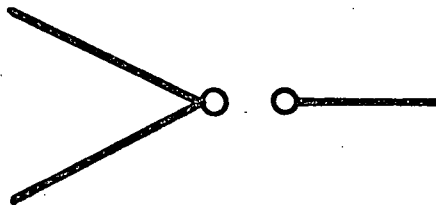
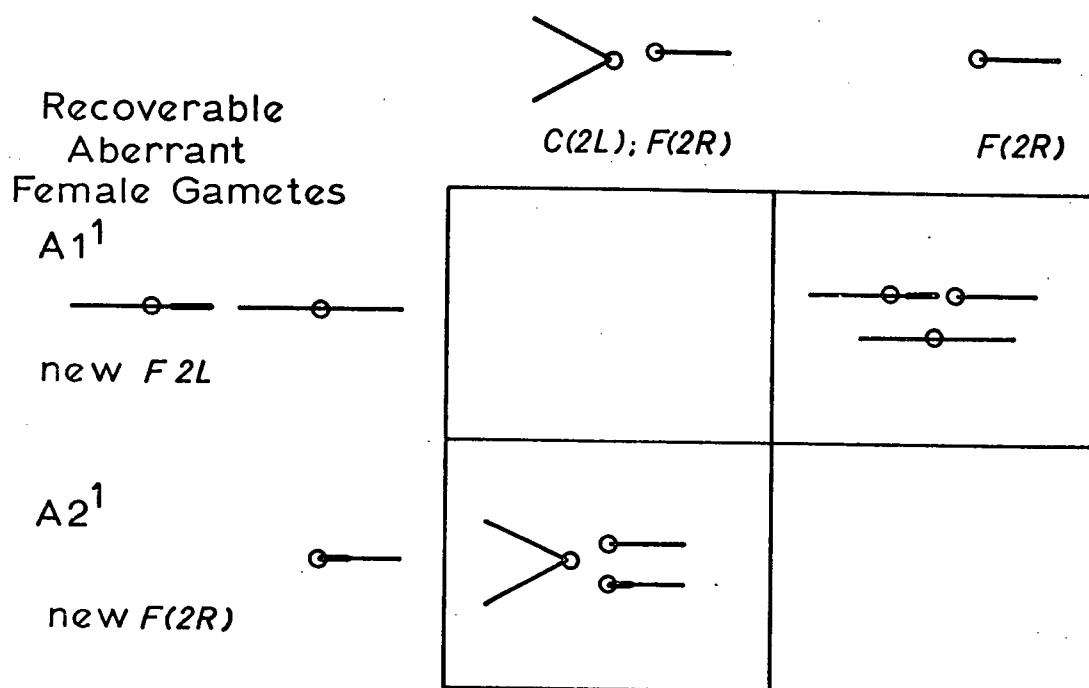


FIGURE 8

The $F(2R)$ males produce two classes of sperm. Consequently, the only recoverable progeny, when mated to females with normal chromosomes, are those from new free arms generated through interchanges. The source of the aberrant chromosome products $A1^1$ and $A2^1$ is shown in Figure 6.

Gametes from $C(2L); F(2R)/F(2R)$ Males

It also provided the means of identifying the other member of the genome involved in the interchange with the second chromosome.

MATERIALS AND METHODS

The symbols used to designate free arms were in keeping with Grell (1970) which is somewhat inconsistent with the recommended terminology (Lindsley and Grell 1968). The left and right centric free arms have been designated F(2L) and F(2R) respectively, followed by the appropriate symbols for any genetic markers they carry. The compound 2L; free-2R stock would be described as C(2L);F(2R)/F(2R) as suggested by Grell (1970).

One combination of chromosomes in the present study has not been previously described. It includes a free-2L chromosome, a free-2R chromosome and an intact chromosome-2 homologue. Such genotypes will be symbolized as F(2L)/In(2LR)SM1,Cy/F(2R) (as they all have been carried over the SM1 balancer chromosome). If the strain is homozygous for both left and right free arms, it will be designated by F(2L)/F(2L);F(2R)/F(2R). Furthermore, the heterologous portion attached to the free arm will be designated by its chromosome number and the arm involved; for example a free-2L derived from an interchange with the right arm of the fourth would be written F(2L;4R).

In the study of free arm recovery, C(2L)lt;F(2R)bw/F(2R)bw males, generated as described by Grell (1970), were mated to In(2LR)SM1,Cy/b pr cn females in bottles. The females were phenotypically Curly (Cy). Each experiment involved approximately 50 bottles with 20 females per bottle. The females were treated with gamma radiation from a ⁶⁰Co source for that time necessary to yield a total dose of 2000 rads. The radiation source was as described in Chapter I.

The few progeny recovered were of three general phenotypes. Progeny

carrying putative new free 2L's had curly wings (Cy) and were of the following genotype F(2L)b pr/In(2LR)SM1,Cy/F(2R)bw. Progeny with new free 2R's expressed two possible phenotypes: lt, which were genotypically C(2L)lt;F(2R)bw/F(2R)cn or +, which were genotypically C(2L)lt;F(2R)bw/F(2R)Dp(2L)lt⁺,cn. The last class was the result of an interchange between the left arm of chromosome 2, distal to the lt locus, and some other chromosome. Thus F(2R)'s that carried a duplication for the left arm were immediately identifiable as being capped rather than captured (compare product A1¹ to A2¹ in Figure 6). Many of the + flies were sterile, some of which expressed the phenotypes of either metamales (X/Y;2/2/2;3/3/3;4/4/4) or intersexes (X/X;2/2/2;3/3/3;4/4/4).

The phenotypically classified free arms were analysed in the following manner. Progeny with a putative F(2L) were mated to flies carrying In(2LR)SM1,Cy/In(2LR)bw^{V1}, a double balancer for chromosome 2. From each line crosses were made between F₁ Cy males and females to test the viability of the homozygous F(2L). In an attempt to determine whether F(2L)'s were capped, approximately 2000 virgin females from each F(2L)b pr/In(2LR)SM1,Cy/F(2L)bw line were mass mated to F(2L)nub b pr/F(2L)nub b pr;C(2R)r1 cn males. Rare nondisjunctional oocytes carrying only newly generated F(2L) chromosomes were rescued by F(2L)nub b pr;C(2R)r1 cn sperm. If the F(2L) carried a duplication of the right arm which covered r1, the progeny would be phenotypically b pr cn. Such F(2L)'s were then classified as being capped. Progeny that were b pr r1 cn could not be immediately classified as a break could have occurred between r1 and the centromere in which case the F(2L) would be capped but not identifiable by this test.

In one experiment the treated females as well as having the marked seconds, b pr cn/In(2LR)SML,Cy, also had marked fourths, one with cubitus interruptus dominant (ci^D) and the other with eyeless dominant (ey^D); each of these are recessive lethals. This was an attempt to determine the involvement of the fourth in quasibivalent formation leading to the generation of F(2L)'s and F(2R)'s. The recovery of free arms was so low and the viability of those recovered so poor, that I did not continue the use of these marked fourth chromosomes.

As an alternative, the new F(2L)'s were mated to a stock that was heterozygous for a chromosome-2 inversion and homozygous for the recessive marker sparkling-poliert (spa^{pol}) on the fourth chromosome. The F₁ progeny were then carried through a test cross. The absence of the expression of spa^{pol} in any of the progeny carrying the newly generated free arm was taken as evidence for the involvement of the fourth chromosome in the formation of the free arm. Upon analysis, I later learned that the absence of this expression could lead to a false interpretation.

The F(2R)'s were mated to C(2L)dp;F(2R)bw/bw. The phenotypically dp + flies from this cross were mated to determine if the F(2R)'s were homozygous viable in which case one would recover phenotypically dp cn flies.

The use of the C(2L)lt in the male parents, for the recovery of newly generated F(2R), provided the means of immediately identifying any new F(2R)'s that had a break point distal to the lt⁺ locus on the interchange chromosome and thus were identified as capped. Those with more proximal break points could not be classified.

A final attempt was made to determine the involvement of the fourths in

the formation of free arms of chromosome 2. The fourths in the free arm strain were made homozygous for the recessive markers ci ey^R svⁿ (ci - cubitus interruptus, ey^R - eyeless-Russian, svⁿ - shaven-naked). The absence of expression of any of the recessive markers was interpreted as an interchange involving chromosome 4.

All viable lines confirmed to carry free arms were examined cytologically in an attempt to determine, from polytene chromosomes, the break points on chromosome 2 as well as on the other chromosome involved in the interchange.

RESULTS

Consistent with the prediction of the quasibivalent model, both F(2L) and F(2R) chromosomes were recovered (Table XV). The theory of equal recovery obviously was not realized in any of the three experiments and the accumulated results show an approximate five-fold greater recovery of F(2R)'s relative to F(2L)'s.

The new F(2L)'s were analysed in various ways in an attempt to determine the origin of the centromere and thereby to classify as capped or captured. It was thought that the ability to render the free arm homozygous might be a preliminary means to determine whether the F(2L) was capped. Table XVI shows that seven of the ten F(2L)'s recovered were homozygous viable. However, this is not a particularly reliable test as F(2L)V17 was not homozygous viable and yet by another means was shown to be capped. F(2L)'s were then tested for a duplication of the right arm by constructing a stock with a F(2L) of known properties (F(2L)nub b pr, obtained from Oak-Ridge Laboratories) and a compound-2R homozygous for rl and cn. The rl locus has been shown to occupy a position within right heterochromatin of chromosome 2 (Hilliker and Holm 1975). The absence of a rl phenotype revealed that the break point, resulting in the formation of F(2L), was distal to the rl⁺ locus in the right arm and thus confirmed that the F(2L) was capped. Of the six F(2L)'s that could be tested in this manner, that is they were successfully incorporated into a C(2R) line, only one carried a rl⁺ duplication (Table XVI).

A third approach to analysing captured vs. capped F(2L)'s was to determine if any of the interchanges involved the X chromosome. The X chromosome being acrocentric implied that, if it were involved in an interchange, the

TABLE XV

Number of induced $F(2L)$'s and $F(2R)$'s recovered from $In(2LR)SM\bar{L},Cy/b\ pr\ cn$ or $In(2LR)SM\bar{L},Cy/Df(2R)M-S2^{10}$ females mated to $C(2L)VH2,1t;F(2R)bw/F(2R)bw$ males.

Female genotype	Number of $F(2L)$	Number of $F(2R)$
$In(2LR)SM\bar{L},Cy/b\ pr\ cn$	7	20
$In(2LR)SM\bar{L},Cy/Df(2R)M-S2^{10}$	1	8
$In(2LR)SM\bar{L},Cy/b\ pr\ cn$	2	19

TABLE XVI

Genetic and cytological properties of induced free-2L chromosomes.

Code	<i>Dp(2R)r1⁺</i>	Homozygous viable	Interchange Chromosome	Break points ***	Capped vs. captured
V2 ²	0 *	+	X	hetero;hetero	capped **
V9 ¹	-	+	X	hetero;hetero	capped **
V17	-	-	X	hetero;hetero	capped **
V46	+	+	4	hetero;hetero	capped **
V7	0	+	X	hetero;hetero	capped **
V9 ²	-	+	X	hetero;hetero	capped **
V31	-	+	4	hetero;hetero	capped **
W18	0	-	unknown	hetero;hetero	unknown ****
Y32	-	+	4	hetero;hetero	unknown
Y42	0	-	2L	44C5-6;23D3-6	capped **

* An "0" indicates the line was not tested.

** Basis for the classification is given in the text.

*** Heterochromatic break points were assumed based on the cytological observation of unaltered euchromatin in polytene chromosomes.

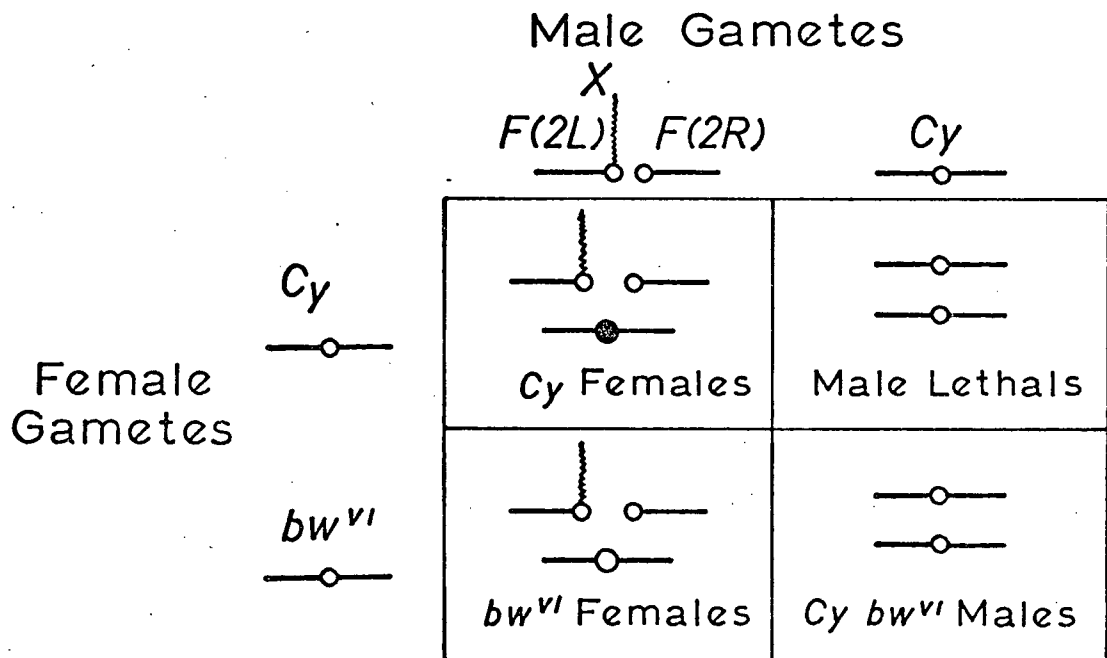
**** This free-2L chromosome, generated from an interchange of the *Df(2R)M-S2¹⁰* chromosome, appears to be an example of a captured rather than a capped free arm.

product would most likely carry the centromere of the second. Products of interchange between chromosome 2 were detected in two ways. The discovery of F(2L)'s that could be made homozygous only in females provided a direct genetic method of determining the involvement of the X chromosome. A second method was employed for those free arms which could not be made homozygous. Males of the genotype F(2L)b pr/In(2LR)SML,Cy/F(2R)bw, were mated to In(2LR)bw^{V1}/In(2LR)SML,Cy females. This cross should have given rise to progeny of three phenotypes: Cy bw^{V1}, Cy and bw^{V1} in both the male and the female progeny. The presence of only Cy bw^{V1} males and only Cy or bw^{V1} females was taken as confirmation of an interchange between the second and the X chromosome (Figure 9). As shown in Table XVI, five of the ten left free arms recovered were involved with the X chromosome, and therefore, were probably capped. Four of these interchange products were homozygous viable; one was not. Interestingly, all the X-2L interchange chromosomes were recovered from the same experiment.

Further classification of free left arms was made through cytological examinations of polytene chromosomes. Nine of the ten appeared perfectly normal, that is no euchromatic rearrangements in the polytenes could be identified, thereby indicating that interchanges took place in the heterochromatic regions. This supports the earlier work of Parker and McCrone (1958) who thought most interchanges were heterochromatic in origin. The one exception in this study had a break in the right arm of chromosome 2 at 44C5-6 of Bridges' map (see Lindsley and Grell 1968) which was capped by the tip of 2L broken at position 23D3-6. Since the homologue, In(2LR)SML,Cy, over which the free arm was generated carried an inversion with a break point distal to 23D3-6, the interchange was recognized as having taken place between

FIGURE 9

Males bearing the $2L \cdot X$ chromosome produce two classes of sperm recoverable when mated to females carrying standard second chromosomes. Since the X is attached to one arm of the second, i.e. $2L;X$ translocation, the free arms of chromosome 2 will be recovered from this cross only in females. The male progeny will inherit the standard second carrying the dominant marker Cy . Note that the Cy/Cy class in males are not recovered owing to a recessive lethal linked to this inverted chromosome.



the arms of sister chromatids.

From the ten recovered left fragments, seven were confirmed to be capped rather than captured by one of the means described above, excluding the test for homozygous viability. Of the remaining three, one was classified as being capped from the test to determine the involvement of the fourth chromosome in the formation of free arms of chromosome 2.

The fourths in the free arm strain were made homozygous for the recessive markers ci ey^R svⁿ. The absence of expression of any of these markers in the F(2L) strain indicated the involvement of the fourth in the interchange. Such was the case with chromosomes F(2L)V31, F(2L)V46 and F(2L)Y32, although several anomalies were noted in the progeny from the stock carrying F(2L)V31. From this stock twenty-six percent (73/277) of the progeny did not express the more distal marker svⁿ but did express the more proximal markers ci ey^R. A subsequent cross between males and females carrying F(2L)V31 and heterozygous for the ci ey^R svⁿ also yielded anomalous results. In this case twenty-nine percent (23/79) did not fall into the expected classes: 11/23 expressed only ci ey^R, 4/23 only expressed ey^R svⁿ, 7/23 only ci and 1/23 expressed ey^R. Such results might be explained by position effect as has previously been shown for ci (Parker 1965, 1967), although such events have not been reported previously for ey^R and svⁿ. Assuming these unusual progeny are the result of position effect would imply that the euchromatic component of chromosome 4 had been transposed to a new heterochromatic site, i.e. the 2R heterochromatin, and thus the F(2L) was probably capped.

The F(2R)'s were grouped into those not carrying the 1t⁺ duplication from the right arm and those with the duplication. Of the 15 fragment rights

without the duplication (Table XVII), 13 were fertile and therefore analysed. Of these 13, seven were homozygous viable. The only method of determining whether they were capped or captured was by cytological means. All had normal polytene chromosomes and thus the other interchange member could not be identified. Again the break point of the right free arms as well as that of the interchange heterologue must have been heterochromatic. Because of the lack of a genetic system and the absence of cytological alterations in the polytene chromosomes of these fragments they could not be classified as capped or captured.

By virtue of the fact that the remaining progeny (32 in total) carried the $1t^+$ marker, located in the 2L heterochromatin (Hilliker and Holm 1975), they were putatively classified as carrying newly generated F(2R)'s. This was confirmed upon subsequent matings with flies of the genotype C(2L)dp;F(2R)be/F(2R)bw. Unfortunately, many of this class were sterile and therefore a distinction between free arm formation and some aneuploid state could not be differentiated; all such flies were not included in Table XVIII. In general, the viability of the $1t^+$ F(2R) stocks was very low. Many stocks died before they could be tested beyond confirming that they carried a new F(2R) (13 out of 32). Of those tested, all 19 were homozygous lethal. Where possible these free arms were also examined cytologically. Of the 12 examined, five appeared normal cytologically, indicating heterochromatic breaks on both members of the quasibivalent. Where the breaks leading to F(2R) formation were in the euchromatin of the left arm of chromosome 2, and therefore recognizable, the break in the other chromosome involved in the interchange was invariably euchromatic. The captured euchromatic component, as indicated in Table XVIII, included 2L, 2R, 3L, 3R and 4R. Interestingly, no such euchromatic interchanges involving the X chromosome were identified.

TABLE XVII

Genetic and cytological properties of induced free-2R chromosomes not carrying the *Dp(2L)lt⁺*.

Code	Homozygous viable	Interchange chromosome	Break points**	Capped vs. captured
V8	+	unknown	hetero;hetero	unknown
V14	+	unknown	hetero;hetero	unknown
V18	-	unknown	hetero;hetero	unknown
V30	-	unknown	hetero;hetero	unknown
V36 ²	-	unknown	hetero;hetero	unknown
V43	+	unknown	hetero;hetero	unknown
W16	-	unknown	hetero;hetero	unknown
Y7	+	unknown	hetero;hetero	unknown
Y37	+	unknown	hetero;hetero	unknown
Y45 ²	-	unknown	hetero;hetero	unknown
Y45 ³	-	unknown	hetero;hetero	unknown
Y51	0	0	0	0
Y58 ³	+	unknown	hetero;hetero	unknown
Y60	+	unknown	hetero;hetero	unknown
Y68 ³	0	0	0	0

* An "0" indicates the line was not tested.

** Heterochromatic break points were assumed based on the cytological observation of unaltered euchromatin in polytene chromosomes.

TABLE XVIII

Genetic and cytological properties of induced free-2R chromosomes carrying *Dp(2L)lt⁺*.

Code	Homozygous viable	Interchange chromosome	Break points***	Capped vs. captured
V1	0*	0	0	capped
V2	0	0	0	capped
V4	-	0	0	capped
V5 ³	0	0	0	capped
V6	-	0	0	capped
V7 ²	0	0	0	capped
V11 ³	0	0	0	capped
V11 ⁴	0	0	0	capped
V13	-	0	0	capped
V32	-	0	0	capped
V36	-	unknown	hetero;hetero	capped
V37	-	unknown	hetero;hetero	capped
V39	0	0	0	capped
V44	0	0	0	capped
W1	0	0	0	capped
W11	-	unknown	hetero;hetero	capped
W14	-	0	0	capped
W20	-	0	0	capped
W23	-	0	0	capped
W32 ²	0	0	0	capped
W41	0	0	0	capped
Y2 ²	-	telomere	34D;telomere	capped
Y8 ¹	0	0	0	capped
Y8 ²	-	3L	36F;61D	capped
Y16	-	2R	38E;32C**	capped
Y28	-	3R	37;99	capped
Y34	-	2L	40A4-5;22B8-9	capped
Y41	-	4R	35A;102E3-4	capped
Y49	0	unknown	hetero;hetero	capped
Y55	-	0	0	capped
Y58	-	2L	39C;26D	capped
Y68	-	unknown	hetero;hetero	capped

* An "0" indicates the line was not tested.

** Capped by the arm of *In(2LR)Cy,SM1* that carries the telomere of 2R.

*** Heterochromatic break points were assumed based on the cytological observation of unaltered euchromatin in polytene chromosomes.

DISCUSSION

The attempt to recover left and right free arms of chromosome 2 was conducted to lend support to the theory that quasibivalent interchanges give rise to nondisjunction and chromosome loss. As predicted, free arms were generated and recovered by using the appropriate selective system.

The free-2L's, ten in total, first were analysed to determine whether they were capped or captured. Through the genetic identification of the presence of a duplication for the rl locus, which is located to the right of the centromere, cytological examinations of salivary gland polytene chromosomes, the genetic recognition of viable interchange products involving the X chromosome and position effect variegation, eight of the ten fragments were classified as being capped. The origin of the other two was indefinite.

The analysis of the F(2L) chromosomes further included an attempt to determine if the fourth chromosome was the other member of the quasibivalent. The fourth chromosome recessive marker, spa^{pol}, was made homozygous in each of the free-2L lines. The absence of expression of this marker in a F(2L) line was expected to genetically identify the fourth as the chromosome involved. Surprisingly, this method indicated that nine of the ten left arm fragments arose from interchange with the fourth, i.e. F(2L;4R). However, five of the nine had previously been confirmed, by genetic means, to have been F(2L;X) interchanges. The fourth, therefore, definitely could not be involved unless each of the fragment formation events had been very complicated multichromosome interchange products. Although such events have been reported (Williamson and Parker 1968) they cannot conceivably explain this inordinately high rate of recovery. It would appear that, as a more likely explanation, spa^{pol}, like spa, is suppressed by an increase in the amount of heterochromatin (Morgan

1947). Furthermore, Morgan showed that deficiencies for the heterochromatin of the right arm of chromosome 2 enhanced the expression of spa. As the heterochromatic effects on spa^{pol} appear to be the same as on spa the present findings indicate that the F(2L) chromosomes were capped, as captured seconds would be partially deficient for the heterochromatin of chromosome-2L and carry no heterochromatin of 2R. It seems most probable that all the F(2L)'s, involving the X chromosome, were capped by the X. Consequently, these interchange chromosomes would carry duplications for the 2R heterochromatin but at the same time be deficient for varying amounts of the X heterochromatin. Results from the present study lend further support to this concept as F(2L)Y42 was generated from an euchromatic interchange with its sister chromatid, thus it definitely carried a duplication for heterochromatic-2R. This free arm suppressed spa^{pol}. Conversely, F(2L)W18 generated from a Df(2R)M-S2¹⁰ chromosome, which is deficient for most if not all of the 2R heterochromatin (Morgan *et al.* 1940, Hilliker and Holm 1975), was the only F(2L) which did not suppress spa^{pol}. In fact, the expression of spa^{pol} was enhanced in the presence of this free left arm.

Because of the unreliability of spa^{pol} owing to its suppression by excess 2R heterochromatin, four F(2L)'s were tested for their involvement with the fourth chromosome, detected by the inability to recover the expression of three different recessive markers on the fourth chromosome. Of the four lines tested, three were confirmed interchanges between a second and fourth chromosome, that is the products were 2L·4R. However, in each case the results revealed anomalies. Two of the lines, V46 and Y32, gave the expected classes Cy/bw^{V1}; ci ey^R svⁿ, Cy and bw^{V1}. The absence of Cy;ci ey^R svⁿ and bw^{V1}; ci ey^R svⁿ served to identify the involvement of the fourth. Although the above classes were expected, one class was missing, namely Cy/bw^{V1} flies

that were wild type for markers on chromosome-4. This class should have been heterozygous for the recessive markers having received a wild type fourth from the free arm parent. This result implies that in these two lines there was only one free marked fourth, the wild type fourth being attached to the F-2L. Consequently, the wild type fourths would never be recovered in the Cy/bw^{V1} progeny and thus all these progeny would be Cy/bw^{V1};ci ey^R svⁿ, receiving only the free fourth from the free arm parent. The question can then be asked: how would a stock be maintained with only a single free fourth? One possible explanation is that during meiosis the 2L·4R chromosome regularly segregated from the free fourth, as well as from In(2LR)SML,Cy thus the In(2LR)SML,Cy chromosome and the free fourth segregated to the same pole. In this way, by mating F(2L)b pr/In(2LR)SML,Cy/F(2R)bw males and females, the stock would maintain a single free fourth. The third fragment (V31), also identified as an interchange with the fourth, differed from the two previously mentioned F(2L) lines in two aspects: 1) this line produced Cy/bw^{V1} phenotypes indicating there were two free fourths in the F(2L) line, one wild type fourth and the other carrying the recessive markers ci ey^R svⁿ, 2) of the F(2L) progeny recovered, twenty-six percent (73/277) were homozygous for only the two most proximal markers, ci and ey^R; svⁿ was not expressed. The loss of ci and ey^R could have been due to some type of position effect suppression of the two proximal genes as has previously been reported for ci (Parker 1965; Parker 1967; Spofford 1976) or could have been the expression of an interchange in which only the 4R distal to ey^R was translocated to F(2L). However, cytological studies did not support the second possibility. A further examination of this strain, by crossing F(2L)b pr/bw^{V1}/F(2R)bw;ci ey^R svⁿ males and females, resulted in the recovery of the two expected chromosome-2 classes, bw^{V1} and b pr bw (the latter class is the expression of both homo-

zygous F(2L);F(2R) chromosomes). None of the regular progeny expressed all three recessive markers on the fourth as the F(2L;4R) carried the wild type alleles. However, from this cross seventeen percent (24/143) of the progeny expressed the ci ey^R phenotypes but not the svⁿ, as found in the previous case, and 12 progeny showed other variations of expression of the three genes: four had only the ey^R svⁿ phenotypes, seven had only ci phenotype and one expressed only the ey^R phenotype. These findings further point out the necessity of caution in the use of a marked fourth chromosome in the analysis of chromosomal interchanges. These results simply report a strange phenomenon, further experiments are required to better determine the cause of these results.

The fourth line tested, W18, indicated the fourth chromosome was not involved in the free arm formation as progeny with the free arm expressed all three recessive genes on the fourth. As this free arm was generated from a chromosome deficient for 2L heterochromatin (Df(2R)M-52¹⁰) and as the polytene preparations appeared normal and as it enhanced the expression of spa^{pol}, this chromosome was possibly a captured F(2L).

The model of quasibivalent formation followed by interchange resulting in nondisjunction is supported by the present data. The recovery of F(2L)'s, with the selection system used, required that the homologue of the second involved in the interchange also be included in the gamete with the free arm. In studies of nondisjunction it is vital that this class include only those cases where homologous centromeres go to the same pole. Thus in this study, as in previous studies using acrocentric chromosomes, nondisjunction is not necessarily synonymous with matroclinous progeny. An interchange can lead to the exchange of centromeres in which case matroclinous progeny would actually be the consequence of disjunction rather than nondisjunction (e.g. B2¹ Figure

6). In acrocentric chromosomes, i.e. 4ths and X's, a progeny matroclinous for the X chromosome could involve a normal X and an interchange X. The interchange X may carry the centromere of the 4th in which case the matroclinous progeny would result from disjunction rather than nondisjunction. With metacentric chromosomes, nondisjunction per se is synonymous with matroclinous progeny. Furthermore, interchange mediated nondisjunction also can not be substantiated unless the chromosome in question (i.e. X or 4th) can be confirmed as being capped. However, from the present study, with the free arms generated from natural metacentric chromosomes, confirmation of the centromeres involved demonstrated that interchanges lead to the nondisjunction of homologous centromeres.

In total, 47 confirmed F(2R)'s were recovered of which 32 were definitely capped as they carried the 1t⁺ marker from the 2L heterochromatin (Hilliker and Holm 1975). The remaining 15 were classified as unknown as they could not be tested for the absence or presence of the centromere of chromosome 2. Thus from the 57 induced fragments produced and tested (both left and right arms), seventy percent (40/57) were confirmed as being capped, i.e. they carried the centromere of chromosome 2.

In conjunction with the Novitski (1951, 1967) "drag hypothesis", the quasibivalent model of nondisjunction predicts a preferential recovery of the shorter chromatid of that chromosome 2 involved in the interchange. It follows, therefore, that the excess recovery of patroclinous over matroclinous progeny, which was previously called chromosome loss, is a function of the preferential recovery of the shorter chromatid as a direct result of quasibivalent interchange. This would also imply the preferential recovery of a capped rather than a captured interchange chromatid. The results,

obtained in the present study, are consistent with this model. I do not mean to suggest, however, that the "drag" effect is an exclusive mechanism for the production of excess nullo-2 eggs, but rather that it contributes to this class of exceptional products.

In view of the quasibivalent model, my original prediction was that the number of left and right free arms generated would be equal. This definitely was not the case as only ten lefts were recovered as compared to 47 rights. There are two possible explanations for this discrepancy. The first suggests either that the other chromosome involved in quasibivalent formation preferentially pairs with the left arm or that interchanges occur here more often. The second possible explanation is that the quasibivalent frequently results in the formation of a trivalent. This would include both seconds and the heterologous chromosome involved in the interchange. The latter explanation agrees with the results of Williamson (1974a) who reported preferential segregation of the X and Y chromosomes in a compound-X-bearing female, even though the X had been involved in an interchange with chromosome-4. In the present study the two second chromosomes regularly would be members of the distributive pool as the balancer, In(2LR)Cy,SML, would prevent crossovers and according to the size rule would pair distributively. An interchange could result in the formation of both left and right free arms, but as the homologues would usually disjoin, more gametes would contain only a free arm (either left or right) than a free arm accompanied by a normal homologue. The present study supports previous observations that the interchange chromosomes separate at meiosis I (Parker 1969; Busby 1971; Parker and Williamson 1970). However, it is possible that the two second chromosomes pair prior to an interchange between one of the second chromosomes and some other heterolo-

logue. The pairing of the homologues possibly guarantees their separation at meiosis I and, similarly, the interchange causes the two interchange products to segregate. If segregation were to occur, gamete $B1^1$ would be recovered more frequently than gamete $A1^1$ (Figure 6). Thus when mated to C(2L);F(2R)/F(2R) more right free arms than left free arms would be recovered, bearing in mind the interchanges leading to free right arms must take place to the left of the centromere in chromosome 2, not as depicted in Figure 6 where the breaks are shown on the right arm. The type of sperm available from the C(2R);F(2R)bw/F(2R)bw males could recover either induced left or right free arms. Nevertheless, the rescuing of a left free arm can only occur if it is recovered with a normal homologue as well. But if the second chromosomes usually segregate, the higher incidence of recovery of right free arms over left would be explained.

In previous studies, in the absence of a free Y chromosome, detachment products of the compound X usually involved chromosome 4 (Parker and Hammond 1957; Parker 1969; Parker and Williamson 1970). As the 4ths are normally members of the distributive pairing pool they would be likely candidates for interchange with other chromosomes entering the pool. From a total of 57 fragments, the other chromosome involved in the interchange was identifiable by genetic or cytological means in 16 cases. Of these only four were confirmed as being the fourth. The other identifiable interchanges included the left arm of the X and both left and right euchromatic portions of chromosome 2 and chromosome 3. However, a very important observation was made concerning the break points. In the X fragments, in which the break in chromosome 2 was euchromatic, the break in the capping segment was also euchromatic. Similarly, in the 27 cases in which the chromosome-2 free arm break point was evidently in heterochromatin, the capping segment also

appeared to have its break in heterochromatin as the polytene chromosomes appeared normal. Moreover, 14 of these 27 interchange products were homozygous viable. This finding suggests that possibly euchromatic breaks can rejoin only with euchromatic breaks and likewise, heterochromatic breaks can rejoin only with heterochromatic breaks. While no exceptions to this last point were witnessed in the present study, one such exception, a quasireciprocal translocation involving C(2L);C(2R) and a standard third was recovered by Hilliker and Holm (1975). Further studies are required to provide more information about the temporal and structural organization of chromosomes during meiosis.

Because of the previous observations, it can be implied that in those free arms in which normal cytological preparations were observed the other chromosome involved was probably the fourth. Involvement with any other chromosome except an entire X (of which five were identified) would be lethal.

Many of the euchromatic breaks provide evidence that very large duplications of the autosomes are tolerated. I also observed, in the crosses between normal females and free arm stocks, almost as many pupae cases as in normal crosses, although only the rare nondisjunctional progeny eclosed.

General Discussion Of Interchange: One of the overall objectives of the present study was to recover and analyse aberrant products of chromosome 2 induced during meiosis. The total number of induced aberrations in chromosome 2, recovered when mated to compound-2 males, was very high and included four classes of gametes: nullo 2, diplo 2, compound-2L and compound-2R. For example, In(2L + 2R)Cy/ap^{wow} females treated with 2000 rads produced 529 recovered aberrant events. Those recovered represent approximately a total of one quarter of those induced as only one quarter of the sperm were capable of rescuing each of the four aberrant classes. Thus, the estimated number of actual aberrations generated was 2,116. This represented 2.98 percent of an estimated total number of 71,000 progeny. It must be noted, however, that this represents only the damage induced and recoverable for chromosome 2 by using the compound-2 selective system. The total chromosomal damage to the entire genome based on the recoverable aberrant events that were recovered from chromosome 2 would be considerable. The above estimates do not take into consideration translocations which are not recoverable except possibly with males bearing free arms. Nevertheless, even then many are lost due to segmental aneuploidy.

The results suggest there are two main factors affecting the recovery of induced aberrations. The most important variable is the isosequentiality of the chromosomes involved. Alterations such as inversions greatly increase the recovery of some exceptional meiotic events. Although of less significance, but still a recognizable factor, is the genetic background of the stocks used. The 1t pk cn stock, for example, was less sensitive to the effects of radiation in regards to the four classes of apparent products recovered, as well as the rate of induced recessive lethals, supporting the

results of Sobels (1971) on stock radiation resistance or sensitivity.

Furthermore, I believe that three of the classes of simultaneously recovered progeny arise from a common mechanism of induction; a mechanism which also accounts for free arm formation. Compound chromosomes, matroclinous progeny, and some patroclinous progeny as well as free arm formation, require two independent breaks occurring at the level of the chromatid. The location of the breaks, the position of the chromatids, and the method of reconstitution determine the recoverable events. It is assumed that many induced breaks will be reconstituted to their original condition. The events recorded here are the result of abnormal associations of these breaks, i.e. interchanges.

Intrachromosomal Breaks: Abnormal reconstitution could occur between breaks within a chromosome, resulting in a variety of rearrangements. The breaks could lead to sister compound chromosome formation, providing one break occurred in each chromatid, and the breaks were on opposite sides of the centromere. Two breaks followed by such reconstitution leading to the formation of a compound chromosome could be referred to as an interbrachial interchange as suggested by Williamson (1969) in the formation of isomarked Y chromosome fragments.

Although not observed in these experiments, intrachromatid breaks resulted in ring chromosomes (Hilliker, personal communication) by the rejoining of breaks on opposite sides of the centromere of a single chromatid. The heterochromatic left and right breaks joined forming a heterochromatic ring, cytologically identified in patroclinous progeny.

If the two breaks occur on the same side of the centromere, two conse-

quences are possible. The adjacent arms could rejoin forming an acentric and a dicentric isochromosome. This would involve a dicentric bridge at anaphase II, possibly resulting in a nullo egg recovered as a patroclinous progeny (Traut 1968). The other alternative is the rejoining of opposite arms forming two normal chromosomes bearing either small duplications or deficiencies, i.e. an induced sister chromatid crossover event.

Interhomologue Breaks: Simultaneous breaks also may occur in chromatids of homologues. The repair of these breaks can result in non-sister compound formation in a manner similar to that previously described for sister compound chromosome formation. If the homologues are heterozygous for a paracentric inversion and if the breaks result in an induced heterochromatic exchange accompanied by an exchange within the inversion, a dicentric bridge would occur at meiosis II which might lead to the production of a nullo 2 egg (Novitski 1955; Ptashne 1960). Induced exchanges did occur as evidenced by the recovery of four wild type progeny from In(2L + 2R)Cy/ap^{wow} females. None were observed from the In(2LR)Cy,SM1/ap^{wow} females presumably as induced crossing over would only lead to segmental aneuploidy owing to the pericentric nature of the inversion. The results (Table V) indicate a significantly lower recovery of induced patroclinous progeny from the latter class, lending support to this notion.

Although, Sturtevant and Beadle (1936) demonstrated double dicentric bridges of the X chromosome lead to nullo eggs recovered as patroclinous progeny, Novitski (1952) found this was true only for acrocentric chromosomes. When the X chromosome had an arm attached (i.e. from the Y chromosome) fewer patroclinous progeny were recovered. Novitski (1955) speculated that the formation of a submetacentric chromosome changed the strength of

the centromere such that the dicentric bridge would fracture resulting in fragments. This would lead to dominant lethals rather than nullo-X eggs. In view of Novitski's finding with a modified X (by the attachment of a Y) breaks between homologues could result in dicentric bridges as well as non-sister compound chromosomes. The dicentric formed in this fashion would result in strong centromeres which would possibly break the bridge. When mated to males carrying compound-2 chromosomes, the bridge products would be lost.

Interchromosomal Breaks: A third type of event could occur between nonhomologous chromosomes and are referred to as interchanges by Parker (1969). As suggested by Parker, interchanges between two nonhomologues of unequal size produce heteromorphic dyads resulting in chromatids of different length attached to the same centromere. As a consequence of interchange, the heterologues segregate at meiosis I, which would result in nondisjunction providing the homologue of one of the interchange chromosomes goes to the same pole. The heteromorphic dyad supposedly orients chromatids of different lengths in such a way as to place the shorter chromatid at the pole as suggested by the "drag hypothesis" (Novitski 1951, 1967) and thus increases the probability of its incorporation into the pronucleus. Assuming, in the present study, that the fourth chromosome was most frequently involved with the second in the formation of a heteromorphic dyad, the results should most often result in the production of patroclinous progeny.

Interchanges: If I extend the use of the term interchange to include breaks in any pair of chromatids, homologues or nonhomologues, it could account for the various meiotic products recovered in immature oocytes, depending upon the manner in which the broken chromatids are rejoined. Such

interchanges could result in both the formation of sister and the formation of non-sister compound chromosomes. The interchange, if between heterologues, could lead to the nondisjunction of a pair of chromosomes and be observed, in the present study, as matroclinous progeny. The reciprocal product of the interchange between heterologues would produce an equal number of nullo eggs, observed as patroclinous progeny, but if the dyad so formed is heteromorphic, i.e. chromatids of different length, it would result in the greater recovery of patroclinous progeny because of the preferential inclusion of the shorter chromatids. Interchange could also result in dicentric formation which may also contribute to this last class. Although the evidence is minimal, interchanges may also be responsible for induced crossing over in heterochromatin. Finally, interchanges can result in the recovery of a half-translocation (which now more suitably might be expressed as one-half the product of a reciprocal translocation) as observed in the free arm studies.

The generation and analysis of interchange events point out four major features: 1) if it is assumed that nondisjunction and chromosome loss are interchange mediated then interchange is increased in the presence of structural heterologues, 2) it also follows from the results of this study that the genetic backgrounds affect the frequency of interchange, 3) probably any interchange can be induced providing an appropriate selective system is available to recover the event, and 4) interchanges appear to occur primarily between euchromatic-euchromatic or heterochromatic-heterochromatic breaks.

CHAPTER V

AN ASSAY FOR THE PRODUCTS
OF CHEMICAL MUTAGENESIS

INTRODUCTION

Chemically induced chromosomal aberrations are of interest to geneticist as well as environmentalists. The increasing numbers of new chemicals appearing in the environment increases the need for sensitive and reliable assay systems to detect chemically induced genetic damage. The identification of mutagens, the genetic manifestation of their action, and the frequency of induced aberrancies are all specific aspects to be considered.

Chemically induced genetic damage may range from toxic lethality, to much more subtle changes such as specific substitutions of the nucleotides in DNA. The former is a very gross analysis and eliminates all levels of damage below total lethality. Different chemical agents have been found to vary greatly in their toxicity as compared with their mutagenic activity. For example, ethyl methane sulfonate (EMS), although highly mutagenic, has been reported to have little effect on viability of bacteria (Loveless and Howarth, 1959). Such findings pose two main questions concerning assays for induced genetic damage: what organism should be used, and what level of damage should be used as the criterion for mutagenicity?

Before these two questions can be answered, it is necessary to look at characteristics of a good assay system. It should 1) be economical 2) be reliable 3) yield relatively fast results 4) depend upon objective interpretation 5) cover a wide range of damage, and 6) involve an in vivo system.

Although various eukaryotic organisms could be used, all having certain advantages and disadvantages, the fruit fly, Drosophila melanogaster, best fits the requirements for an initial assay system for environmental mutagens. Flies can be reared more economically and in larger numbers than most other

multicellular organisms. Their short life cycle, of approximately 12 days, yields rapid results, and the wealth of accumulated information on *Drosophila* greatly facilitates interpretation of the results. Finally, the simplicity of the *Drosophila* genome, is also an asset as there are only four linkage groups.

The second question, the level of damage used as a criterion, must be concerned with the genetic material. A good assay system must be able to detect a wide range of damage. Ideally, it should reveal point mutations, chromosomal aberrations and abnormal meiotic behaviour of the chromosomes.

Parker (1968), Sobels (1971) and Abrahamson and Lewis (1971) have reviewed the various methods of determining genetic damage in *Drosophila* and the advantages of this organism. Parker (1968) suggests two distinct approaches towards the study of induced radiation damage during meiosis: either measure the frequency of the damage or gain insight into the mechanism of meiosis.

Most of the assay systems thus far developed have focused on the X and/or Y chromosome (Valencia 1970). As indicated by Parker (1968), studies may fail to detect induced genetic damage not because it did not occur but because an appropriate selective system, which avoided dominant lethality, was not available. This problem was more readily resolved with the sex chromosomes than with the autosomes, and for this reason they have received the greatest attention.

With the use of the multiplier and appropriate selective systems, I was able to extend experimental approaches that previously had been restricted to the sex chromosomes. I was able to detect and quantify simultaneously four

classes of structural or numerical autosomal aberrations during meiosis in females as well as recessive lethals via means of the internal dosimeter test. The multiplier system served as an internal dosimeter by testing for recessive lethals.

Having developed an assay system for autosomes, using gamma radiation as a mutagenic agent, I tested the system with known chemical mutagens to determine whether or not they were radiomimetic.

Two known mutagenic compounds were tested: ethyl methane sulfonate (EMS) and mitomycin C (MC). The former is a monofunctional alkylating agent and the latter may act either as a monofunctional or as a bifunctional alkylating agent. The molecular mechanisms of these chemicals are not completely known, but it is thought that they react at the N-7 position of guanine, resulting in the excision of the nitrogenous base from the DNA molecule (for a review see Drake 1969; Freese 1963). It is not the purpose of this paper to examine the effect of alkylating agents at the molecular level, but rather to determine the general effect of these chemicals on chromosomes. The type of damage caused by the alkylating agents should be elucidated by the recovery of some or all of the four classes of aberrations discussed in previous chapters.

One of the questions concerning alkylating agents is their ability to induce chromosomal breakage. Friston (1970) reported the recovery of EMS induced breaks. Additionally, Williamson (1970b), treating male *Drosophila*, reported the recovery of two deletions within the Y chromosome but Lim and Snyder (1968) recovered only one translocation out of 2,000 sperm tested. On the other hand, Cattanaach and Williams (1971) did not recover in mice, EMS induced translocations or fragments when they treated spermatogonia. How-

ever, Cattanaach, Pollard and Isaacson (1968) did recover EMS induced breaks in postmeiotic cells of mice.

More recently, Hilliker (1976), using EMS, induced 113 mutations in the heterochromatic region of chromosome 2 of Drosophila melanogaster. Analysis of these mutations indicated that none were deletions. This finding was in keeping with the results of Lim and Snyder (1974), who found no deletions out of 82 induced mutations in the zeste-white region of the X chromosome. Further evidence indicating the inability of EMS to produce chromosomal breaks was reported by Schewe et al. (1971a), who failed to recover X-Y or Y-Y interchanges when treating female *Drosophila*. In general, the results from mutagenic studies on EMS indicated that it does not cause chromosomal breakage, although the evidence was not conclusive.

MC, a bifunctional alkylating agent, appeared to cause chromosomal breaks. Natarajan and Schmid (1971) observed chromosomal aberrations induced by MC in Chinese Hamster cells, mainly in constitutive heterochromatin. Similar results obtained from studies in plants have been reported by Rao and Natarajan (1967). Furthermore, Schewe et al. (1971b) recovered X-Y and Y-Y, MC-induced interchanges both in female and in male *Drosophila*.

Several studies have been directed towards the effect of alkylating agents on nondisjunction. At the time, nondisjunction was usually considered to be independent of any interchange event, and the results like those from studies on chemical induced breakage, have been inconclusive. Hayashi and Suzuki (1970) reported increased nondisjunction of the X chromosomes in females of *Drosophila* when treated with MC, which is in contrast with the results of Schewe et al. (1971b). Moreover, Schewe et al. (1971a) did not

find EMS to have any measurable effect on nondisjunction.

Because of the interchange nature of the aberrant meiotic products recovered from radiation treated females, the assay system provides a means of determining whether the alkylating agents are capable of causing similar effects. As well, the comparative recovery of the various classes should confirm whether or not the aberrant events are exclusively break induced.

The assay system provides a means of examining more extensively some of the effects of known mutagens as well as testing new environmental chemicals. The present system, because of its many advantages, should be able to extend studies on mutagenic agents. For example, it allows mutagenic studies of a broader nature to be conducted on autosomes, rather than being restricted to sex chromosomes. It also allows for the simultaneous recovery of four different classes or progeny. The selective exclusion of all regular meiotic products facilitates the recovery of a relatively larger number of aberrant eggs without massive and laborious screening techniques. Finally, the multiplier system enables the calculation of the frequency of each event and acts as an internal dosimeter to lend credence to negative results.

MATERIALS AND METHODS

The stocks used for this study and the mating protocol was the same as that presented in Chapter I. Virgin females, aged three days, were fed in bottles for 24 hours on a solution of 0.025M EMS in one percent sucrose (Lewis and Bacher 1968) or 125ug/ml MC in one percent sucrose, then allowed to recover for 24 hours before being mated to compound-2 males. A recessive lethal test on the X chromosome (as described in Chapter I) was conducted for each experiment. The exceptional progeny were not tested beyond confirmation of their original classification.

RESULTS

As recorded in Table XXI, the mean number of progeny per vial was significantly reduced by the administering of alkylating agents, the reduction being greater for MC than for EMS. Although the mean number of flies per multiplier vial differed, the variances were homogeneous using Bartlett's test of homogeneity of variance for different sample sizes.

Table XX contains the frequencies of the four classes of aberrant chromosome-2 products, as well as the frequency of recessive lethals. These frequencies were calculated using the data recorded in Table XIX. Consistent with all previous experiments, fewer aberrations were recovered from the lt pk cn stock, suggesting that their resistance to radiation possibly also applies to chemical mutagens. This is in agreement with Browning (1970) who found that various strains of *Drosophila* showed different sensitivities to EMS induced recessive lethals.

The system definitely indicates differences between the monofunctional and bifunctional alkylating agents. While EMS failed to induce compound chromosomes, one was recovered from the In(2L + 2R)Cy/ap^{wow} strain and nine from the +/lt pk cn strain when they were treated with MC (Table XIX). More compounds were recovered from the structural homozygotes than from inversion heterozygotes; a finding that was in keeping with those made in the radiation studies. Interestingly, the nine compounds generated from +/lt pk cn were all sister-strand attachments of the lt pk cn chromosome, one left and eight rights. Each of these compounds represented independent events as each was derived from a different female.

TABLE XIX

Number of progeny recovered as exceptional chromosome-2 products of meiosis and the number of X-linked recessive lethals obtained in corresponding multiplier tests.

Female genotype	Treatment	Estimated total number of progeny	Number of exceptional chromosome-2 products				Recessive lethals *
			Compounds (2L or 2R)	Matroclinous (Disomic-2 eggs)	Patroclinous (Nullo-2 eggs)	Equational nondisjunctions	
<i>In</i> (2L + 2R) <i>Cy/ap</i> ^{wow}	0	203,000	0	137	220	6	13/1832
<i>In</i> (2L + 2R) <i>Cy/lt pk cn</i>	0	55,800	0	23	38	1	2/941
<i>In</i> (2L + 2R) <i>Cy/ap</i> ^{wow}	EMS	66,600	0	87	114	7	20/837
<i>In</i> (2L + 2R) <i>Cy/lt pk cn</i>	EMS	36,300	0	26	19	0	23/815
<i>In</i> (2L + 2R) <i>Cy/ap</i> ^{wow}	MC	24,700	1	34	29	7	17/717
<i>In</i> (2L + 2R) <i>Cy/lt pk cn</i>	MC	24,000	0	16	8	3	8/603
<i>+/lt pk cn</i>	0	62,600	0	1	5	0	1/977
<i>+/lt pk cn</i>	EMS	42,700	0	13	5	5	20/456
<i>+/lt pk cn</i>	MC	19,400	9	22	5	0	22/615

* Number of X-linked lethals per total number of tested chromosomes obtained from the multiplier test. See text for full description.

TABLE XX

Estimated percent recovery of the four classes of progeny that arose from aberrant chromosome-2 products of meiosis.

Female genotype	Treatment	Estimated frequencies of aberrant chromosome-2 products (in percent)*				
		Compounds	Matroclinous	Patroclinous	Equationals	Recessive lethals**
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	0.000	0.067	0.108	0.003	0.710
<i>In(2L + 2R)Cy/lt pk cn</i>	0	0.000	0.041	0.068	0.002	0.212
<i>In(2L + 2R)Cy/ap^{wow}</i>	EMS	0.000	0.131	0.171	0.011	2.389
<i>In(2L + 2R)Cy/lt pk cn</i>	EMS	0.000	0.072	0.052	0.000	2.822
<i>In(2L + 2R)Cy/ap^{wow}</i>	MC	0.004	0.138	0.117	0.028	2.371
<i>In(2L + 2R)Cy/lt pk cn</i>	MC	0.000	0.067	0.033	0.013	1.327
<i>+/lt pk cn</i>	0	0.000	0.002	0.008	0.000	0.102
<i>+/lt pk cn</i>	EMS	0.000	0.030	0.012	0.012	4.386
<i>+/lt pk cn</i>	MC	0.046	0.113	0.026	0.000	3.577

* Calculations were made using unmodified data.

** Based on the results recorded in Table XIX.

TABLE XXI

Percent matroclinous progeny based on an estimated total number of progeny produced in, and corresponding mean multiplier value obtained for, each of the experiments.

Female genotype	Treatment	Mean number of progeny per multiplier vial \pm S.E.	Estimated total number of progeny	Number of matroclinous progeny (Disomic-2 eggs)	Frequency in percent	95% Confidence interval *
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	104.12 \pm 2.81	203,000	137	0.067	0.057 - 0.080
<i>In(2L + 2R)Cy/lt pk cn</i>	0	63.85 \pm 1.57	55,800	23	0.041	0.026 - 0.062
<i>In(2L + 2R)Cy/ap^{wow}</i>	EMS	74.78 \pm 2.26	66,600	87	0.131	0.105 - 0.161
<i>In(2L + 2R)Cy/lt pk cn</i>	EMS	44.72 \pm 1.39	36,300	26	0.072	0.047 - 0.105
<i>In(2L + 2R)Cy/ap^{wow}</i>	MC	40.27 \pm 3.73	24,700	34	0.138	0.095 - 0.193
<i>In(2L + 2R)Cy/lt pk cn</i>	MC	33.75 \pm 2.40	24,000	16	0.067	0.038 - 0.108
<i>+/lt pk cn</i>	0	71.18 \pm 2.01	62,600	1	0.002	0.000 - 0.009
<i>+/lt pk cn</i>	EMS	56.08 \pm 2.19	42,700	13	0.030	0.016 - 0.052
<i>+/lt pk cn</i>	MC	29.52 \pm 2.06	19,400	22	0.113	0.071 - 0.172

* Based on the table or corrected fiducial limits of expectations from Stevens (1942).

The results recorded in Table XXI indicate that the alkylating agents used significantly increases the recovery of matroclinous progeny from the structural heterozygotes when using the ap^{wow} chromosome. With the lt pk cn structural heterozygotes the results show a moderate increase. This demonstrates the importance of the sensitivity of the strains used in any assay system. Additionally, when comparing the effects of EMS and MC on any given strain of structural heterozygotes, there is no difference. However, when comparing the effects of EMS and MC on the structurally homozygous strain, +/lt pk cn, a far greater increase is observed in the recovery of matroclinous progeny with the MC treatment than with the EMS treatment.

As revealed by the results recorded in Table XXII, the only increase in recovery of patroclinous progeny was obtained from In(2L + 2R)Cy/ap^{wow} females upon treatment with EMS. Since increases in the recovery of matroclinous progeny were taken to be nondisjunctional events, an equal increase was expected for the reciprocal product, namely, patroclinous progeny. However, this was not realized. Consequently, it has not been possible to offer an interpretation to the events giving rise to these exceptional progeny.

The results of the chemical mutagens are not as precise or consistent as those found for gamma radiation. Two factors may account for this variability: 1) it is much more difficult to standardize a chemical treatment than it is to standardize radiation exposure, and 2) alkylating agents greatly reduce viability and increase sterility of *Drosophila* females indicating the biological potency of these chemicals. Thus, these agents affect the genetic material as well as the physiology of the fly. Such variables make it difficult to determine accurately subtle differences. However, this assay system, with a built in dosimeter (Tables XIX and XX), makes negative results

TABLE XXII

Percent patroclinous progeny based on an estimated total number of progeny produced in, and corresponding mean multiplier value obtained for, each of the experiments.

Female genotype	Treatment	Mean number of progeny per multiplier vial \pm S.E.	Estimated total number of progeny	Number of patroclinous progeny (Nullo-2 eggs)	Frequency in percent	95% Confidence intervals*
<i>In(2L + 2R)Cy/ap^{wow}</i>	0	104.12 \pm 2.81	203,000	220	0.108	0.095 - 0.124
<i>In(2L + 2R)Cy/lt pk cn</i>	0	63.85 \pm 1.57	55,800	38	0.068	0.048 - 0.094
<i>In(2L + 2R)Cy/ap^{wow}</i>	EMS	74.78 \pm 2.26	66,600	114	0.171	0.141 - 0.206
<i>In(2L + 2R)Cy/lt pk cn</i>	EMS	44.72 \pm 1.39	36,300	19	0.052	0.032 - 0.082
<i>In(2L + 2R)Cy/ap^{wow}</i>	MC	40.27 \pm 3.73	24,700	29	0.117	0.079 - 0.169
<i>In(2L + 2R)Cy/lt pk cn</i>	MC	33.75 \pm 2.40	24,000	8	0.033	0.014 - 0.065
<i>+/lt pk cn</i>	0	71.18 \pm 2.01	62,600	5	0.008	0.003 - 0.019
<i>+/lt pk cn</i>	EMS	56.08 \pm 2.19	42,700	5	0.012	0.004 - 0.027
<i>+/lt pk cn</i>	MC	29.52 \pm 2.06	19,400	5	0.026	0.008 - 0.059

* Based on the table or corrected fiducial limits of expectation from Stevens (1942).

more meaningful as the recessive lethal test provides confirmation that the mutagen penetrated the target cells.

A note of caution must be made concerning comparisons. One assumption required for comparisons between any agents, whether radiation, chemical and/or control, is that oocytes of similar maturity are being treated and recovered. This makes comparisons between the chemical mutagens and radiation difficult. The radiation treatment of 2000 rads effectively destroys ninety percent or more of the most mature (stage 14) oocytes. Thus, radiation induced aberrations basically represent stage 7, or immature, oocytes. The chemical mutagens in these experiments presumably will affect both stages. Thus the comparison of results between the two treatments may not be that meaningful. There may also be a residual effect of these chemicals as possibly indicated by the increased frequency of equational nondisjunctions recovered in four of the experiments as shown in Tables XIX and XX.

DISCUSSION

The assay system used in this study, including the internal dosimeter, is an effective means of testing the consequences of exposing female *Drosophila* to chemical mutagens. The system, via the broad spectrum of recoverable events, provides for an analysis of the types of damage inflicted by a mutagen.

Chapter II provided evidence that compound chromosome formation is a translocation event, or more specifically, an interchange event between sister or non-sister chromatids. The interchange event requires two independent breaks which rejoin in the appropriate way to form a new compound chromosome. The recovery of ten compound chromosomes from MC treated females and none from the EMS treated females supports the findings of Cattanaach and Williams (1971), Hilliker and Holm (1975), Lim and Synder (1974), Natarajan and Schmid (1971), Schewe, Suzuki and Erasmus (1971a,b) that EMS does not induce chromosomal rearrangements. In the previous chapter I discussed three classes of interchange: intrachromosomal, interhomologous and interchromosomal. The fact that all ten compound chromosomes recovered from the MC treated females were sister-strand attachments implies that this chemical can cause breaks, but it would appear that the interchanges are restricted to the intrachromosomal type.

Based on the above evidence that MC, but not EMS, induces breaks, one would expect a higher recovery of matroclinous progeny from the former, assuming nondisjunction is caused by interchanges. The results in Table XXI indicate that both alkylating agents caused a significant increase over the controls in the recovery of matroclinous progeny from structural heterologues.

A comparison of the frequencies of recovery of matroclinous progeny, between In(2L + 2R)Cy/ap^{wow} treated with EMS and with MC revealed no significant difference. Moreover, there is no significant difference between In(2L + 2R)Cy/1t pk cn when treated with the two alkylating agents, although this strain shows an overall lower frequency. This failure of MC to increase the frequency of matroclinous progeny over EMS may suggest that although MC can cause interchanges, for unknown reasons, the interchanges are limited to the intrachromosomal type (as indicated by the results of compound chromosome formation) thus interchromosomal interchanges leading to nondisjunction do not occur. Furthermore, not all nondisjunction may be interchange mediated. The mechanism by which nondisjunction is induced by alkylating agents should be investigated further.

The frequency of recovery of patroclinous progeny as recorded in Table XX for structural heterologues was not significantly greater than the frequency of recovery of matroclinous progeny when treated with either alkylating agent with the exception of In(2L + 2R)Cy/ap^{wow} when treated with EMS. Moreover, the frequency of patroclinous progeny, except for the one noted, was not greater than the frequency of the controls. If the control values had been ignored these results would have suggested that the patroclinous progeny were the reciprocal product of nondisjunction, i.e. that is no chromosome loss. However, in view of the control values, it is difficult to accept this interpretation. It would appear, nevertheless, that chemically induced nondisjunction is not interchange mediated as, according to the "drag hypothesis", one would expect a preferential recovery of the nullo eggs, which would be observed as a higher recovery of patroclinous progeny.

These studies on the effect of alkylating agents upon the formation of

aberrant chromosome-2 products of meiosis are only preliminary. However, they do demonstrate a method by which the developed assay system can not only detect the effect of mutagens, but also provide an approach to disclose the mechanism of their action.

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